Oxygen Binding to a Model for the Active Site in Cobalt-Substituted Hemoglobin

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Abstract: Cobalt(II) porphyrin complexes, which are models for the active site in cobalt-substituted hemoglobin, CoHb, have been synthesized and characterized by NMR and visible spectroscopy. The model compounds are based on the tetratolylporphyrin ring system and have covalently attached chains which terminate in a pyridyl ring. The cobalt(II) complexes of these "looping-over porphyrins" react reversibly with dioxygen at low temperatures. The oxygen affinities of these compounds have been measured and the results compared with those obtained with other porphyrins and the protein. Our results indicate that there is negligible enhancement of the oxygen affinity of a cobalt(II) porphyrin when an axial base is covalently attached.

In recent years our understanding of the factors controlling the binding of molecular oxygen to hemoglobin and myoglobin (Hb,Mb)[2] has greatly increased. Syntheses of models for the active sites in these proteins have allowed the mode of oxygen binding to be established[3] and have demonstrated the role of the hydrophobic pocket in stabilizing the heme–dioxygen complex.[4,5] We have felt that the preparation of model compounds would be greatly aided if the tetraarylporphyrin ring system could be selectively functionalized. To this end we have developed a synthetic route to monosubstituted tetraarylporphyrins.[6] In this paper we wish to report the functionalization of the latter compounds to yield models for the active site in Mb. The compounds prepared in this work are sketched in Figure 1.

In these compounds the axial base is covalently linked to the porphyrin by an ether-type linkage. We have investigated the importance of this linkage and its length by studying the oxygen affinity of the cobalt(II) complexes of these porphyrins. The choice of cobalt, rather than iron, was made for two reasons: (1) more extensive thermodynamic data are available for related cobalt systems;[7-14] and (2) studies of the cobalt systems allow further tests of differing hypotheses[15,16] concerning the nature of the cooperativity of oxygen binding in cobalt-substituted and natural hemoglobins. We have previously shown that cobalt(II) porphyrins bind oxygen reversibly.[10] Hoffman and co-workers[7,8] have shown that cobalt reconstituted hemoglobin and myoglobin (CoHb, CoMb) are reversible oxygen carriers and that CoHb exhibits the same allosteric linkages as normal hemoglobin. Maxwell et al.[17] have shown that the infrared stretching frequencies of bound dioxygen are essentially the same in both HbO_2 and $CoHbO_2$. Collman et al.[18] have documented the same fact for simple metalloporphyrins. It is now clear that both the Co and Fe dioxygen complexes are best formulated as $M^{III}O_2^-\cdot$, in line with the original proposal of Weiss[19] and later that of Hoffman et al.[20] The cobalt porphyrins and globins differ from their iron-containing counterparts in that the former have a somewhat lower affinity for dioxygen because of a smaller enthalpic contribution to the overall free energy change. The results of our previous studies of these cobalt–dioxygen complexes have recently been reviewed.[21]

Results and Discussion

The looping-over porphyrins used in this work have an ether type linkage for the covalent attachment of the axial base. The general method of synthesis involves the reaction of an appropriately substituted alkyl bromide with the phenolic porphyrin, 5-(2-hydroxyphenyl)-10,15,20-tritolylporphyrin (**5**). As an example, the synthesis of porphyrin **2** is outlined in Figure 2. Methyl 5-bromopentanoate is stirred with **5** in the presence of solid potassium carbonate to give the ester **6**. Hydrolysis of the ester with alcoholic sodium hydroxide gives the acid **7**, which is converted to the acid chloride and then condensed with 3-aminopyridine to give **2** in 27% yield. The NMR spectrum of **2** is shown in Figure 3. The cobalt complex may be prepared by refluxing the porphyrin with an excess of cobalt acetate in a chloroform–acetic acid mixture. The insertion reaction must be carried out in the absence of air to prevent the rapid oxidation of the Co(II) to Co(III).

Upon exposure to dioxygen, at low temperatures, methylene chloride solutions of these five-coordinate Co(II) porphyrins exhibit a change in the visible spectrum (Figure 4), which is a function of the dioxygen pressure. Removal of the dioxygen restores the original spectrum. We have previously assigned this spectral change to the formation of a 1:1 dioxygen complex[10] and have described in detail the evaluation of the equilibrium constant.[22]

The three porphyrins **2**, **3**, and **4** prepared in this work have side chains of different lengths. Molecular models show that coordination of the pyridine ring to the central cobalt atom is extremely difficult in compound **4**, less difficult in **3**, and relatively unstrained in **2**. The oxygen affinity of the compounds would be expected to parallel this trend. In order to determine the effect, if any, of the covalent attachment of the axial base on the oxygen affinity of these cobalt porphyrins, we have also studied the oxygenation of Co(TPP)(3-Mepy). The bulk of the axial base in this complex should be about equal to that found in compounds **2**, **3**, and **4**.

The results of our equilibrium studies are shown in Tables I and II. Also listed in Table I are values of the thermodynamic parameters previously reported for Co(PPIX-DME),[10,22] CoMb,[8] and CoHb.[14] Inspection of the values of log K for the three compounds Co(TTP)(3-Mepy), **2**, and **3** in methylene chloride shows that the oxygen affinities do vary slightly, as one would expect, on stereochemical grounds. Similarly, there is a dependence of the equilibrium constant on the nature of the solvent; the more polar medium, CH_2Cl_2, favors oxygen uptake.[11]

The lower oxygen affinity of the cobalt complex of **3** is consistent with the strain involved in the coordination of the axial base in this compound. Inspection of space-filling molecular models indicates that the propoxy chain in this compound is rather short to form a strong bond from the nitrogen atom to the cobalt atom. We conclude that a six-coordinate dioxygen adduct should be relatively unstable. This conclusion is further supported by our work with the 2-(3-pyridyl)acetoxy derivative, compound **4**. We have found that the cobalt complex of this porphyrin behaves as if intermolecular coordination of axial bases is favored over intramolecular coordination. Such a result is consistent with our previous findings that sterically

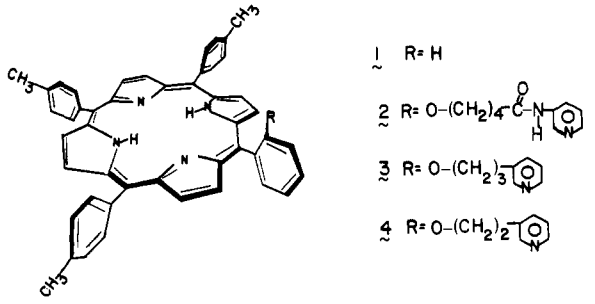
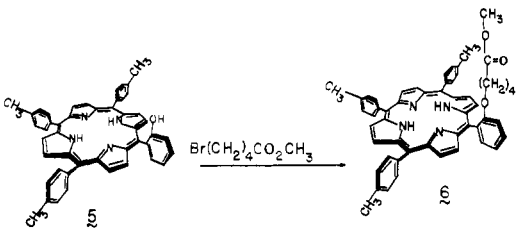


Figure 1. The porphyrins **2**, **3**, and **4** were synthesized in this work. The synthesis of R = OH (**5**) was reported earlier (ref 6).



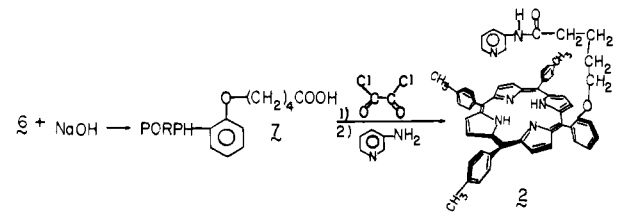


Figure 2. Scheme for the synthesis of 5-[2-(4-(3-pyridylcarbamide)-*n*-butoxy)phenyl]-10,15,20-tritolylporphyrin (**2**).

hindered bases will not bind to Co(II) porphyrins.[23]

The thermodynamic data shown in Table I do not allow any firm conclusions to be drawn. The limited temperature range over which these compounds can be studied makes an analysis of the differences in ΔH and ΔS difficult. Within experimental error, these data are consistent with an extrapolated value of $\Delta G°_{298}$ of 5.8 ± 1.0 kcal/mol, a value which applies to all model porphyrin compounds reported in the literature.

The covalent attachment of an unstrained axial base has demonstrably little effect on the oxygen affinity of cobalt porphyrins. We would like to point out that this should not be the case for iron porphyrins. The covalent attachment of an axial base to a metalloporphyrin serves to assure, within the stereochemical limitations of an individual system, that the

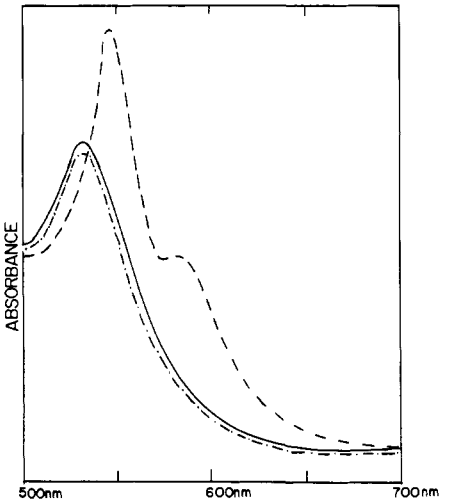


Figure 4. Visible spectrum (−78 °C) of Co[5-(2-(4-(3-pyridylcarbamide)-*n*-butoxy)phenyl)-10,15,20-tritolylporphyrin] (1.5×10^{-5} M) in CH_2Cl_2: (—) under argon; (- - -) under oxygen (1 atm); (- · · · -) under argon after several oxygenation–deoxygenation cycles.

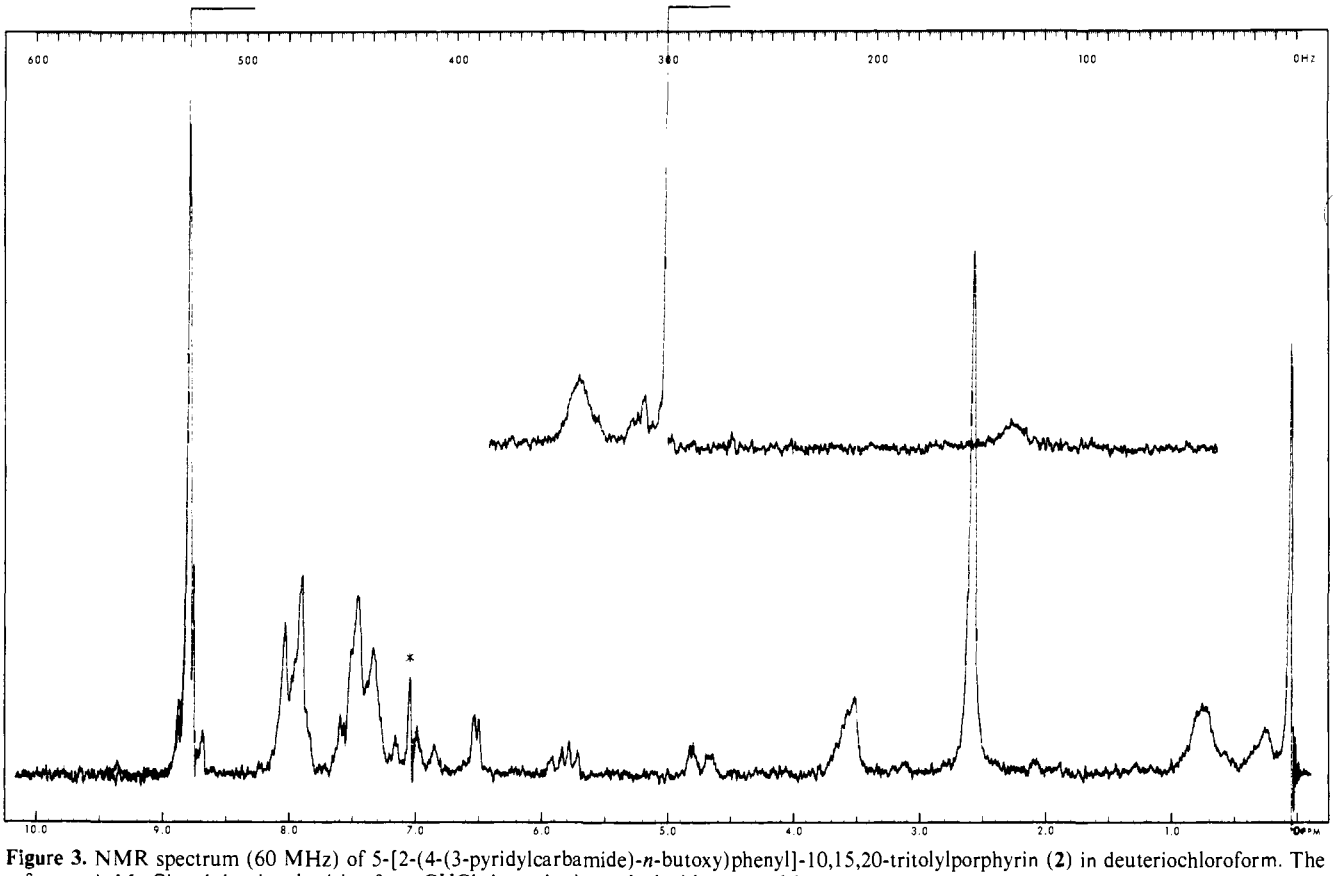


Figure 3. NMR spectrum (60 MHz) of 5-[2-(4-(3-pyridylcarbamide)-*n*-butoxy)phenyl]-10,15,20-tritolylporphyrin (**2**) in deuteriochloroform. The reference is Me_4Si and the signal arising from $CHCl_3$ impurity is marked with an asterisk.

Table I. Thermodynamic Data[a] for the Reversible Binding of O_2 to Co Porphyrins and Proteins

Compd	Solvent	ΔG_{298}, kcal/mol	ΔH, kcal/mol[b]	ΔS, eu[c]
Co(TPP)(3-Mepy)	CH_2Cl_2	5.7	−8.1	−46
2	CH_2Cl_2	4.7	−7.2	−40
2	Toluene	5.1	−5.0	−34
3	CH_2Cl_2	6.1	−8.5	−49
Co(MeO-TPP)(py)	Toluene	7.1	−9.3	−55[d]
Co(PPIX-DME)(py)	Toluene	6.7	−9.2	−53[e,f]
Co(PPIX-DME)(py)	CH_2Cl_2	5.0	−12.0	−57
CoMb horse	H_2O	2.4	−11.3	−46[g]
CoMb whale	H_2O	2.4	−13.4	−53[g]
CoHb	H_2O	2.0	−11.9	−46.6[h]

[a] Standard state of 1 Torr. [b] Estimated standard deviation is 1–2 kcal/mol. [c] Estimated standard deviation is 3–4 eu. [d] Reference 13; stated errors are ±1.1 kcal/mol and 4 eu. [e] It is pleasing to find that T. J. Beugerlsdijk and R. S. Drago (*J. Am. Chem. Soc.*, **97**, 6466 (1975)) have confirmed these values. Their results of $\Delta H = -7.8$ (3) kcal/mol and $\Delta S = -49$ (1.3) eu do not differ significantly from those above. [f] Reference 10, 22. [g] Reference 8. [h] Reference 14.

Table II. Equilibrium Constants for the Reversible Binding of O_2 to Co Porphyrins[a]

Compd	Solvent	Temp, °C	Log K, mm^{-1}
Co(TPP)(3-Mepy)	CH_2Cl_2	−63	−1.67 (11)
Co(TPP)(3-Mepy)	CH_2Cl_2	−53	−2.13 (8)
Co(TPP)(3-Mepy)	CH_2Cl_2	−45	−2.31 (10)
2	CH_2Cl_2	−63	−1.28 (11)
2	CH_2Cl_2	−53	−1.69 (9)
2	CH_2Cl_2	−45	−1.85 (11)
2	Toluene	−78	−1.82 (5)
2	Toluene	−63	−2.19 (10)
2	Toluene	−53	−2.49 (10)
3	CH_2Cl_2	−78	−1.14 (11)
3	CH_2Cl_2	−63	−1.84 (5)
3	CH_2Cl_2	−56	−2.11 (6)
Co(PPIX-DME)(py)	CH_2Cl_2	−37	−1.55 (10)
Co(PPIX-DME)(py)	CH_2Cl_2	−31	−1.75 (10)
Co(PPIX-DME)(py)	CH_2Cl_2	−23	−2.15 (5)

[a] Standard deviations are estimated from the least-squares analysis of the spectra; see ref 22.

metal will be five-coordinate. In such systems the covalent linkage makes the formation of six-coordinate, bis-base complexes unlikely and should favor coordination of dioxygen. One would expect this to be important in determining the magnitude of the equilibrium constants for dioxygen coordination in iron porphyrins because bis-base complexes are greatly favored for ferrous porphyrins. They are disfavored in cobaltous porphyrins. For example, Brault and Rougee[24] have studied the binding of imidazole to Fe(TPP) and report values of 8.8×10^3 and 6.8×10^4 M^{-1} for K_1 and K_2. For comparison, the stepwise formation constants of the 3,4-dimethylpyridine complexes of Co(*p*-MeOTPP) are respectively 10^3 and 10^{-1} M^{-1} according to Walker.[12] These data indicate that the oxygen affinity of a ferrous porphyrin with a covalently attached axial base should be appreciably higher than that of the same porphyrin in solution with a molar excess of base. It appears that one of the functions of the protein is to assure five-, rather than six-coordination at iron in Hb and Mb.

Experimental Section

The NMR spectra were obtained on a Perkin-Elmer R20B or Varian T60 spectrometer operating at 60 MHz. Unless otherwise specified, the solvent was deuteriochloroform with Me_4Si as internal standard. The shifts, δ, are given in parts per million. The spectra were taken on saturated solutions. It should be noted that the shifts are concentration dependent.[25] The protons at the 2, 3, 7, 8, 12, 13, 17, and 18 positions of the porphyrin ring are referred to below as the β-pyrrole protons.

Analyses were performed by Micro-Tech Laboratories, Skokie, Ill. and by the Analytical Services Laboratory, Northwestern University. The porphyrins were analyzed as the cobalt derivatives.

The chromotographic separations described below were effected by the dry-column procedure[26] using either alumina (Fisher Scientific, A-540) or silica gel (Woelm-04526; obtained through ICN Pharmaceuticals, Inc.). The chloroform used as eluent was USP grade, unless otherwise specified.

All experimental operations with Co(II) complexes were carried out under argon in Schlenk-type glassware. Solution spectra in the UV and visible were obtained on a Cary 14 spectrometer in a low-temperature cell as described previously.[10]

Reagents. All solvents used in the oxygen binding studies were deaerated before being used by bubbling prepurified argon through them for at least 1 h. Dichloromethane was distilled from P_2O_5, toluene from Na or Na/K alloy, and 3-methylpyridine and pyridine from KOH. All distillations were carried out under nitrogen. All other reagents were used as supplied unless otherwise noted.

5-[2-(3-Pyridyl)propoxyl]phenyl-10,15,20-tritolylporphyrin (3). In 50 mL of DMF 1.0 g of 5-(2-hydroxyphenyl)-10,15,20-tritolylporphyrin[6] was dissolved and 3.0 g of 3-(3-bromopropyl)pyridine hydrobromide[27] was added. To the solution 8 g of potassium carbonate was added and the mixture stirred at 40 °C for 36 h. Water (100 mL) was then added to precipitate the porphyrin, which was filtered off on a Büchner funnel. The precipitate was washed with 25 mL of water and dried in an oven.

The crude product was dissolved in chloroform and chromatographed on an alumina column (60 × 2 cm). The first band which separates contains the product and unreacted starting material. Some material remains at the top of the column and cannot be removed. The first band was collected and chromatographed on a silica gel column (60 × 2 cm). Upon elution with chloroform two bands separated. The first contained unreacted starting material and the second contained **3.**

The reaction can be followed by TLC on silica using chloroform as the solvent. The product moves slowly, while the starting material moves quickly. The yield was 0.465 g (40% based on 2-hydroxyporphyrin). Anal. Calcd for $C_{55}H_{43}CoN_5O_1$: C, 77.8; H, 5.1; N, 8.2. Found: C, 76.8; H, 5.1; N, 7.8. NMR δ 8.85, 8.80 (ds, 8 H, β-pyrrole), 8.06 (m, 6 H), 7.65 (d, 1 H), 7.45 (d, 6 H), 7.25 (m), 7.10 (m), 7.00 (m), 6.15 (dd), 5.75 (d), 3.70 (t, 2 H, $-OCH_2-$), 2.60 (s, 9 H, $-CH_3$), 1.30 (m), −2.65 (s, 2 H, NH). See Figure 5.

5-[2-(3-Pyridyl)ethoxy]phenyl-10,15,20-tritolylporphyrin (4). The synthesis of the above porphyrin was exactly the same as for **3** except that 3-(2-bromoethyl)pyridine hydrobromide[27] was substituted for 3-(3-bromopropyl)pyridine hydrobromide. The yield was 40% based on 2-hydroxyporphyrin. Anal. Calcd for $C_{54}H_{41}CoN_5O_1$: C, 77.7; H, 5.0; N, 8.4. Found: C, 76.6; H, 5.0; N, 7.5. NMR δ 8.90, 8.75 (s, q, 8 H, β-pyrrole), 8.05 (d, 6 H), 7.65 (d, 1 H), 7.45 (d, 6 H), 7.20 (m), 7.00 (m), 5.10 (d), 4.75 (dd), 3.85 (t, 2 H, $-OCH_2-$), 2.60 (s), 2.00 (t), −2.70 (s, 2 H, NH). See Figure 6.

5-[2-(4-Carbomethoxy)butoxy]phenyl-10,15,20-tritolylporphyrin (6). To a 250-mL round-bottom flask equipped with a magnetic stirrer was added 50 mL of DMF, 1.5 g of 5-(2-hydroxy)phenyl-10,15,20-tritolylporphyrin, and 3.5 g of potassium carbonate. Then 2.8 g of methyl 5-bromovalerate in 5 mL of DMF was added, over a 0.5-h period, to the stirred reactants. The reaction mixture was stirred for 24 h.

The reaction mixture was poured into 200 mL of water and the resulting slurry filtered on a Büchner funnel. The precipitate was

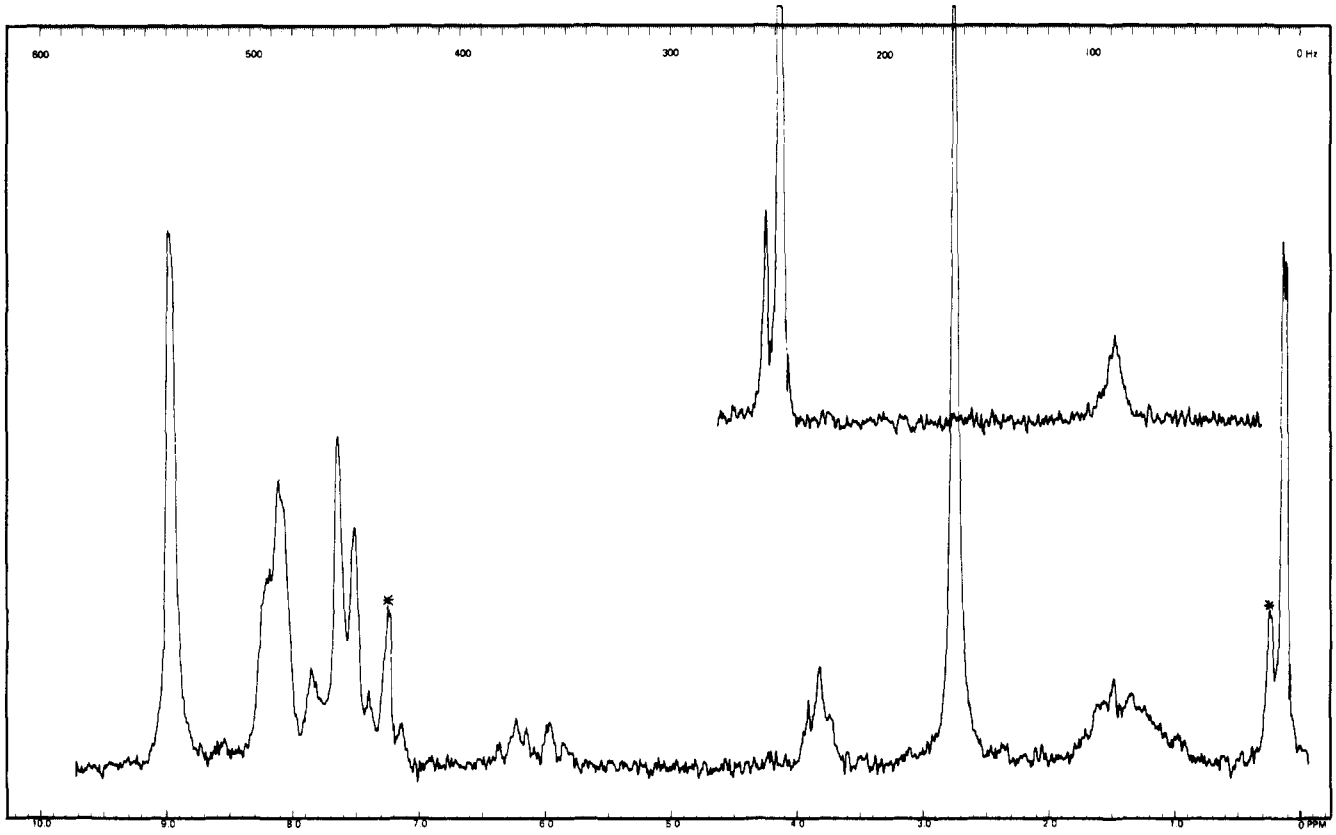


Figure 5. NMR spectrum (60 MHz) of 5-[2-(3-pyridyl)propoxy]phenyl-10,15,20-tritolylporphyrin (**3**) in deuteriochloroform. The reference is Me_4Si and the signals arising from impurities are marked with asterisks.

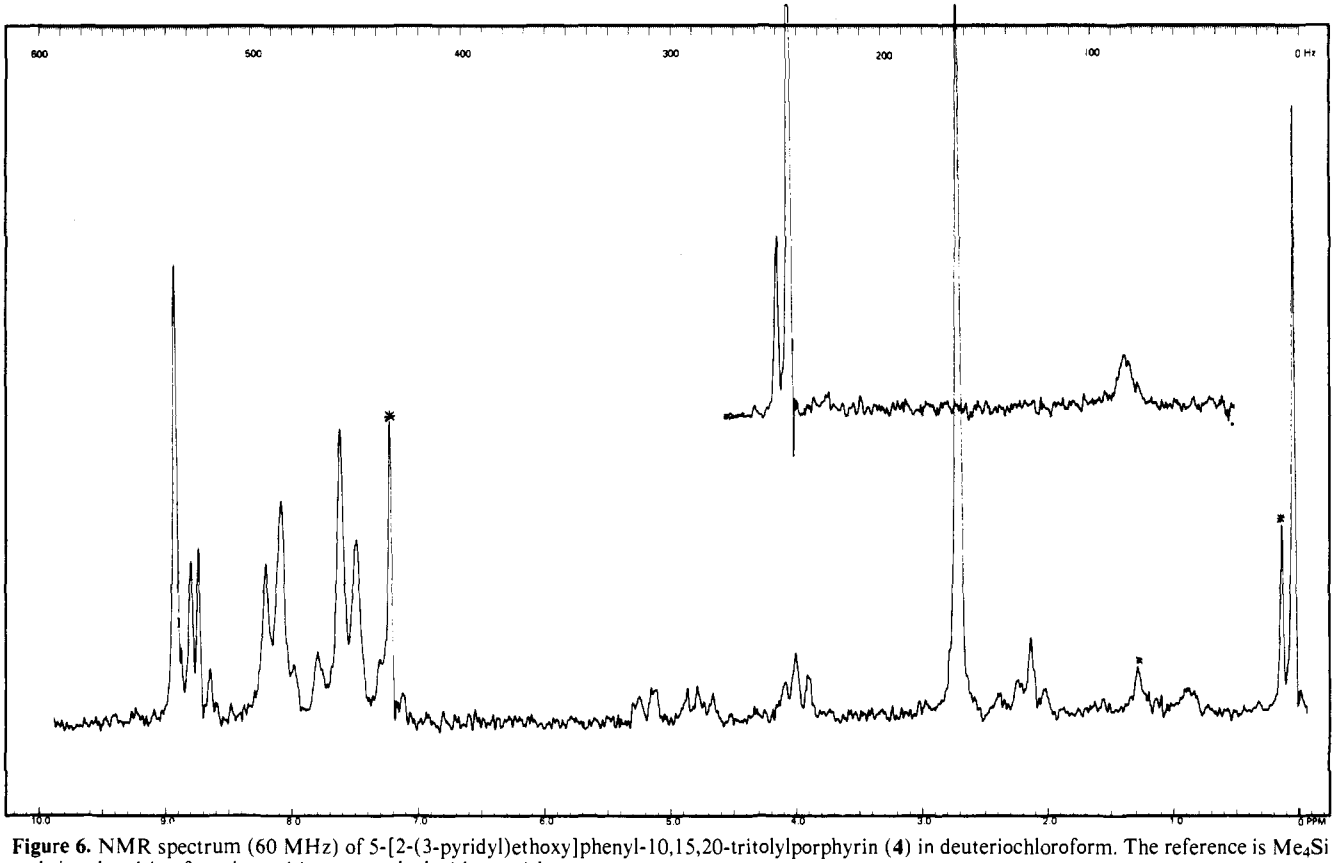


Figure 6. NMR spectrum (60 MHz) of 5-[2-(3-pyridyl)ethoxy]phenyl-10,15,20-tritolylporphyrin (**4**) in deuteriochloroform. The reference is Me_4Si and signals arising from impurities are marked with asterisks.

washed once with 25 mL of water, twice with 5 mL of methanol, and then dried in an oven. This dried material was dissolved in chlorofrom and chromatographed on an alumina column (60 × 2 cm). Any unreacted material appears as a slow moving red band at the top of the column, while the ester moves with the solvent front. The ester band was flashed down and rechromatographed on a silica column (60 × 2 cm). Again chloroform was used as the eluent. A yellow forerun was followed by the ester band. The solution of the ester was flashed down

to dryness, leaving a purple mass which can be converted to a fine powder by heating with methanol and then removing the methanol under vacuum.

The reaction can be followed by TLC on alumina using toluene as the solvent. The ester moves quickly, while the hydroxyporphyrin moves very slowly. The yield was 1.5 g (86% based on 2-hydroxyporphyrin): NMR δ 8.80 (s, 6 H, β-pyrrole), 7.93 (d, 6 H), 7.35 (d, 6 H), 7.20 (s), 7.03 (s), 3.75 (t, 2 H, $-OCH_2-$), 2.90 (s, 3 H, methyl), 2.55 (s, 9 H, methyl), 1.36 (t, 3 H), 0.85 (m, 6 H), −2.76 (s, 2 H, pyrrole NH).

5-[2-(Valeroxyl)phenyl]-10,15,20-tritolylporphyrin (7). In 100 mL of THF 2 g of **6** was dissolved and 5 mL of methanolic sodium hydroxide was added. The sodium hydroxide solution was prepared by dissolving 4 g of sodium hydroxide in 5 mL of water and then adding 50 mL of methanol. The reaction mixture was refluxed for 1 h to hydrolyze the ester.

The THF was flashed off and the precipitated porphyrin filtered off and dried. The porphyrin was dissolved in chloroform and chromatographed on a silica gel column (46 × 2 cm). A small band of unhydrolyzed ester moved with the solvent front. The product (acid) moved very slowly down the column. A dark brown band remained at the top of the column. This band was poured off and the column once again eluted with chloroform. The acid band was collected and evaporated to dryness. The yield was 1.32 g (77% based on 2-hydroxyporphyrin).

5-[2-(4-(3-pyridylcarbamide)-*n*-butoxy)phenyl]-10,15,20-tritolylporphyrin (2). To 100 mL of benzene was added 1.32 g of **7** and 2.6 mL of oxalyl chloride. The solution was stirred for 3 h and then taken to dryness on a rotary evaporator. Benzene (100 mL) was added and the solution flashed down again.

The acid chloride was then dissolved in 50 mL of benzene. To this solution was added 3.9 g of 3-aminopyridine in 50 mL of benzene and the mixture refluxed for 4 h.

The reaction mixture was flashed to dryness and the product dissolved in 30–40 mL of chloroform, and then 20–30 mL of dilute acetic acid (3 M) and 20–30 mL of water added. The chloroform was then removed under reduced pressure and the porphyrin precipitated. The porphyrin was filtered off and the above procedure repeated two more times. Finally, the porphyrin was washed with 10 mL of wet methanol and dried.

The solid was dissolved in chloroform and chromatographed on a silica gel column (60 × 2 cm). Three bands separated on the column. A slow moving pink forerun was followed by a slowly moving dark red band. A brown band slowly separated from the tail of the dark red band. A large amount of black material remained at the top of the column. This black material can be poured off and a mixture of ethyl ether–chlorofrom, 20:30, can be used to speed elution.

The dark red band was collected and concentrated. This solution was chromatographed on an alumina column (8 × 2 cm) to remove any acid porphyrin which stuck to the top of the column. The yield was 0.5 g (27%) based on 2-hydroxyporphyrin. Anal. Calcd for $C_{57}H_{46}CoN_6O_2$: C, 75.6; H, 5.1; N, 9.3. Found: C, 74.6; H, 5.1; N, 8.8. NMR δ 8.75 (m, 8 H, β-pyyrole), 7.93 (d, 6 H, tolyl 2,6 protons), 7.55 (d, 1 H), 7.35 (d, 6 H, tolyl 3,5 protons), 7.10 (m), 6.94 (m), 6.80 (m), 6.47 (d, 1 H H_2 pyridyl), 5.76 (dd, 1 H, H_5 pyridyl), 4.67 (dm, 1 H,H_6 pyridyl), 3.5 (m, 1 H, amide), 3.48 (m, 2 H, $-OCH_2-$), 2.55 (s, 9 H, methyl), 0.7 (m, 4 H), 0.2 (m, 2 H), −2.76 (s, 2 H, pyrrole NH). See Figure 6.

Metal Insertion. The metal insertion reaction was similar to that of Rothemund and Menotti[28] with the following modifications. All reactions were carried out under an inert atmosphere, since the five-coordinate cobalt complex immediately oxidizes at room temperature.

In a 250-mL three-neck round-bottom flask were placed 250 mg of porphyrin and 200 mg of cobaltous acetate. To this flask there were added 50 mL of deaerated chloroform and 50 mL of deaerated glacial acetic acid. This mixture was refluxed under nitrogen until absorption bands from the free porphyrin disappeared (about 1.5 h). The solution was cooled and 30–40 mL of deaerated water was added to precipitate the porphyrin. The porphyrin was filtered and dried in vacuo for a few hours.

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References and Notes

(1) (a) Northwestern University; (b) University of Maryland.
(2) Abbreviations used in this paper: Hb = hemoglobin; Mb = myoglobin; L = any nitrogenous base; P = porphyrin; PPIX–DME = protoporphyrin IX dimethyl ester; TTP = 5,10,15,20-tetratolylporphyrin; TPP = 5,10,15,20-tetraphenylporphyrin; *p*-MeOTPP = 5,10,15,20-*p*-methoxytetraphenylporphyrin; 3-Mepy = 3-methylpyridine.
(3) J. P. Collman, R. R. Gagne, C. A. Reed, W. T. Robinson, and G. A. Rodley, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1326 (1974).
(4) J. E. Baldwin and J. Huff, *J. Am. Chem. Soc.*, **95**, 5757 (1973).
(5) J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, **97**, 1427 (1975).
(6) R. G. Little, J. A. Anton, P. A. Loach, and J. A. Ibers, *J. Heterocycl. Chem.*, **12**, 343 (1975). We note that all of the references in Table I of that paper are out of sequence by one digit. Reference 2 should be ref 1, etc.
(7) C. A. Spilburg, B. M. Hoffman, and D. H. Petering, *J. Biol. Chem.*, **247**, 4219 (1972).
(8) G. C. Hsu, C. A. Spilburg, C. Bull, and B. M. Hoffman, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2122 (1972).
(9) D. V. Stynes, H. C. Stynes, B. R. James, and J. A. Ibers, *J. Am. Chem. Soc.*, **95**, 1796 (1973).
(10) H. C. Stynes and J. A. Ibers, *J. Am. Chem. Soc.*, **94**, 1559 (1972).
(11) H. C. Stynes and J. A. Ibers, *J. Am. Chem. Soc.*, **94**, 5125 (1972).
(12) F. A. Walker, *J. Am. Chem. Soc.*, **95**, 1150 (1973).
(13) F. A. Walker, *J. Am. Chem. Soc.*, **95**, 1154 (1973).
(14) T. Yonetani, H. Yamamoto, and G. Woodrow, *J. Biol. Chem.*, **249**, 682 (1974).
(15) J. L. Hoard and W. R. Scheidt, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 3919 (1973); **71**, 1578 (1974).
(16) R. G. Little and J. A. Ibers, *J. Am. Chem. Soc.*, **96**, 4452 (1974).
(17) J. C. Maxwell, J. A. Volpe, C. H. Barlow, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **58**, 166 (1974); J. C. Maxwell and W. S. Caughey, *ibid.*, **60**, 1309 (1974).
(18) J. P. Collman, J. I. Brauman, T. R. Halbert, and K. S. Suslick, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3333 (1976).
(19) J. J. Weiss, *Nature (London)*, **202**, 83 (1964).
(20) B. M. Hoffman, D. L. Diemente, and F. Basolo, *J. Am. Chem. Soc.*, **92**, 61 (1970).
(21) F. Basolo, B. M. Hoffman, and J. A. Ibers, *Acc. Chem. Res.*, **8**, 384 (1975).
(22) J. A. Ibers, D. V. Stynes, H. C. Stynes, and B. R. James, *J. Am. Chem. Soc.*, **96**, 1358 (1974), and references cited therein.
(23) R. G. Little, B. M. Hoffman, and J. A. Ibers, *Bioinorg. Chem.*, **3**, 207–215 (19).
(24) D. Brault and M. Rougee, *Biochem. Biophys. Res. Commun.*, **57**, 654 (1974).
(25) W. S. Caughey, J. D. Alben, W. Y. Fujimoto, and J. L. York, *J. Org. Chem.*, **31**, 2631 (1966).
(26) B. Loev and M. M. Goodman, *Chem. Ind. (London)*, 2026 (1967).
(27) E. M. Hawes and H. L. Davis, *J. Heterocycl. Chem.*, **10** 39 (1973).
(28) P. Rothemund and A. R. Menotti, *J. Am. Chem. Soc.*, **70**, 1808 (1948).