cally accelerated by one-electron oxidants ($K_2S_2O_8$, VO_2^+, $(p\text{-}BrC_6H_4)_3N^{+}\cdot$) without serious effect on product composition. Thus in 70% acid addition of 0.1 molar ratio of $K_2S_2O_8$ leads to complete reaction in 1 h. (iii) Reactions in D_2SO_4/D_2O solutions lead to **5** which contains an N–H (always >80% of expected ^{1}H integral) and which has deuterium incorporated at the α carbon. At least in $K_2S_2O_8$-promoted reactions, there can be extensive α-deuteration. Thus with 0.5 equiv of $K_2S_2O_8$ in 70% D_2SO_4/D_2O, **2** gave **5** with >60% of complete exchange of α-CH_2 groups, the β-CH_2 and N–H of **5** being apparently unaffected. The N–H of **5** therefore comes from an α-CH_2 group by either 1,2 or 1,5 transfer and deuteration at α-CH_2 must occur subsequent to this. The effects of oxidants suggest the involvement of aminium cation radicals, which are known to undergo 1,5-hydrogen atom transfers[12] and also to deprotonate from carbon to form α-amino radicals.[13] The stable radical cation salt, $C_{12}H_{24}N_2BF_4$, from one-electron oxidation of **2**,[4] is quantitatively transformed into a 1:1 mixture of salts **5** and **6** on heating the solid to 130 °C, but is stable in the H_2SO_4 solutions where **5** is formed and indeed acts as an inhibitor of the reaction leading to **5**. The stable radical cation has an *in, in* conformation,[4] whereas models show that the intramolecular transfer of hydrogen inside the cage is only possible from an α-CH_2 next to an outwardly pyramidalized nitrogen. An *in, out* or *planar, out* radical cation is likely to be involved in the thermal decomposition and related intermediates may occur in the reactions in acid solution.

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(6) Based on NMR studies in H_2SO_4/H_2O solution. The second protonation is half-complete in 49.5% H_2SO_4. The log *I* vs. H_0 plot has a slope of 0.9.
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Cytochrome *c* Models

Sir:

The kinetic features of cytochrome *c* have been intensively studied over the last decade[1] establishing the criteria for its outer-sphere electron transfer mechanism, probably via the exposed heme edge.[2,3] By contrast, the thermodynamic and structural features have received less definitive study. We address three questions: (a) what are the structural changes accompanying the redox reaction, (b) is the intrinsic ligation capacity of methionine to iron modified by the protein, and (c) what determines the high redox potential of the Fe(II/III) couple?

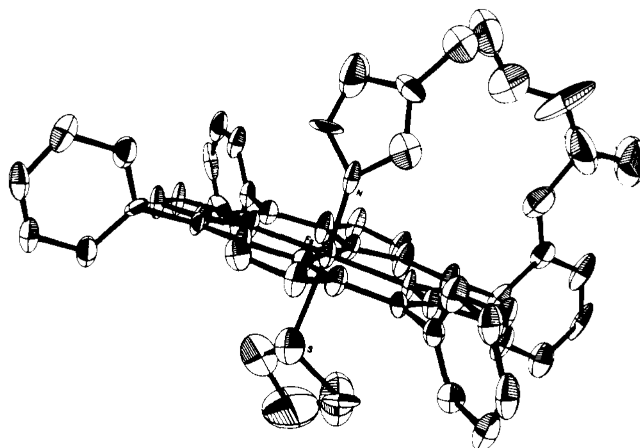

Figure 1. An ORTEP Plot of the Fe($\overline{C_5\text{-Im}}$)(TPP)(THS) molecule **4**. Atoms are represented by their vibrational ellipsoids contoured to enclose 25% of the electron density.

Since the crystal structures of various ferro- and ferricytochromes *c* reveal only very minor protein reorganization upon redox,[2,4] we shift our focus of attention to changes in bond lengths to iron. If small, these can in part rationalize rapid electron transfer (the Franck–Condon principle).[5] Our first goal was to synthetize and structurally characterize a redox pair of low-spin thioether liganded hemes which were identical except for the oxidation state of the iron. The diamagnetic ferrous complex $Fe^{II}(THT)_2(TPP)\cdot THT$ (**1**) was isolated by anaerobic treatment of Fe(TPP)[6] with tetrahydrothiophene (THT).[7] Its exact one-electron oxidation product $[Fe^{III}(THT)_2(TPP)]^+$ can be prepared electrochemically or by treatment of $Fe(OClO_3)(TPP)$[8] with excess THT and isolated as a low-spin perchlorate salt $[Fe(THT)_2(TPP)]ClO_4\cdot 2CHCl_3$ (**2**). That thioethers bind sufficiently strongly to form a six-coordinate complex and that their ligand field strength is sufficiently high to yield a low-spin complex (μ_{eff} = 2.1 BM) is notable. We believe that the notion of methionine as an intrinsically poor ligand to ferric hemes has been exaggerated.[9] In ferricytochrome *c* only moderately strong field and strongly binding ligands (cyanide, imidazole, etc.) displace methionine[10] suggesting that incomplete[11–13] or nonexistent[9] thioether ligation in earlier ferricytochrome *c* models is the result of unrecognized, but recently emphasized,[8] competition from coordinating anions.

The X-ray structures of **1**[14] and **2** have been determined, but crystallographic difficulties forced us to abandon **2** in favor of an analogous pentamethylene sulfide derivative $[Fe(PMS)_2(TPP)]ClO_4\cdot 3CHCl_3$ (**3**).[15] Comparison of the Fe–N bond lengths [Fe^{II}_{av} 1.996 (6); Fe^{III}_{av} 1.982 (6)] reveals distances entirely consistent with expectations based on other low-spin ferrous and ferric hemes.[16] Quite unexpectedly, however, there is virtual parity of the Fe–S bonds in the two oxidation levels (Fe^{II}–S = 2.34 Å; Fe^{III}–S = 2.33, 2.35 Å). Apparently the expected increase in Fe–S upon oxidation due to poor compatibility of the "hard" Fe(III) for the "soft" thioether is offset by the increased charge attraction of Fe(III) for its ligands. This suggests that the same lack of substantial nuclear motion upon redox might also obtain in cytochrome *c*, implying that the choice of methionine as a ligand is beneficial to rapid electron transfer.

More satisfying than the above models is a true synthetic analogue of cytochrome *c* whose synthesis exploits the "tail

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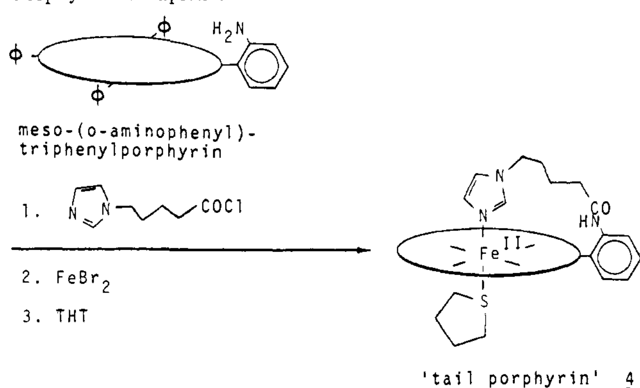
Scheme I. Synthetic Route for the Preparation of the "Tail Porphyrin" Complex 4



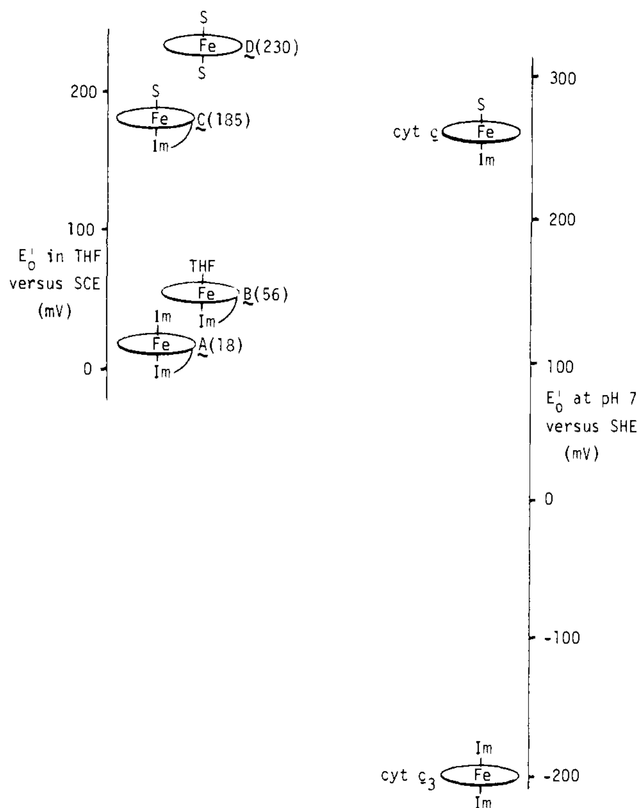


Figure 2. Comparison of the formal potentials, E_0', of the Fe(II/III) couple for synthetic tetraphenylporphyrin complexes A–D (left-hand column) with those of the cytochromes c and c_3 (right-hand column). Compounds 1 and 2 in the text correspond to couple D and compound 4 corresponds to the ferrous component of couple C. The ferrous component of couple A was generated by addition of N-methylimidazole to the "tail porphyrin".

porphyrin" concept.[13] The well-established superiority of imidazoles over thioethers in binding hemes[10,11,13,17] prescribes that the opportunity for more than *mono*imidazole coordination be denied. Treatment of 5-(N-imidazolyl)valeryl chloride with α,β,γ-triphenyl(δ-o-aminophenyl)porphyrin followed by iron incorporation and thioether addition gives, with perseverance, diamagnetic purple crystals of a ferrocytochrome c analogue $Fe^{II}(C_5\text{-Im})(TPP)(THS)\cdot C_6H_6$ (**4**, Scheme I). Its X-ray structure[18] (Figure 1) confirms the formulation. It is not only the first isolated cytochrome c analogue but also the first structurally characterized "tail porphyrin" complex. Currently the Fe-S distance is within experimental error of that in **1** but disorder in the methylene groups and crystal degradation under X-rays severely limit quantitative aspects of the structure. The orientation of the imidazole ring is such that its projection onto the porphyrin plane is ~4° to the nearest N_p–Fe–N_p vector—near the sterically least favored position.[16]

We have used electrochemistry to generate a *ferri*cytochrome c analogue directly from **4** and also to study the effect of axial ligation on the Fe(II/III) couple in a low dielectric medium. Using a system of the type [Pt|THF, Fe(II/III), $Bu_4N^+PF_6^-$|SCE], we have measured formal potentials with all accessible combinations of imidazole and thioether ligation. Controlled potential electrooxidation, voltammetry, and coulometry collectively show well-behaved one-electron changes for all the couples A–D quoted in Figure 2 indicating no significant structural change (i.e., no axial ligand change) between oxidized and reduced forms. The most significant result is that substitution of one imidazole by a thioether (compare couples A and C in Figure 2) results in a 167-mV positive shift. This is in reasonable agreement with the ca. 150-mV shift observed for the same ligand substitution in Harbury's aqueous heme octapeptide system[11] and also in Wilson's mixed solvent study of mesoheme derivatives,[19] suggesting that this axial ligand effect is essentially independent of medium. However, the potential difference between the bis-histidine ligated cytochromes c_3 ($E_0' \simeq -200$ mV vs. SHE)[20] and the common cytochromes c ($E_0' \simeq +260$ mV)[2] is about 460 mV. We conclude that this large difference is due to about 160-mV contribution from histidine replacement by methionine with the remainder (ca. 300 mV) due to environment effects. The latter contribution reflects a destabilization of the ferric form (relative to ferrous) due to the apparent inability of cytochrome c to delocalize the positive charge generated by oxidation of the iron. Kassner[21] has ascribed this to the low dielectric interior of cytochrome c and Stellwagen[22] has emphasized the lack of heme exposure to the aqueous medium. The significant observation[2] that only the cytochromes c have their heme propionic acid side chains buried within the protein (not possible with c_3) suggests that restricted propionate ionization may be a crucial environmental factor.

In summary, isolable well-characterized synthetic models for cytochrome c suggest that (a) Fe–S bond lengths in the hemoprotein will be close to 2.34 Å and insensitive to valency of iron if the protein is unconstrained, (b) thioethers are better ligands to Fe(III) than previously believed, and (c) thioether–imidazole ligation is responsible for about 160-mV shift in the Fe(II/III) redox potential relative to bis-imidazole ligation, independent of environment.

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(15) Crystal data: $FeS_2N_4C_{54}H_{48}ClO_4\cdot 3CHCl_3$, orthorhombic, $P2_12_12_1$, $Z = 4$; $a = 17.830$ (3), $b = 18.781$ (3), $c = 18.187$ (3) Å; $\rho_{calcd} = 1.45$, $\rho_{exptl} = 1.45$ g/cm^3; 5778 unique observed data, $R = 0.077$.
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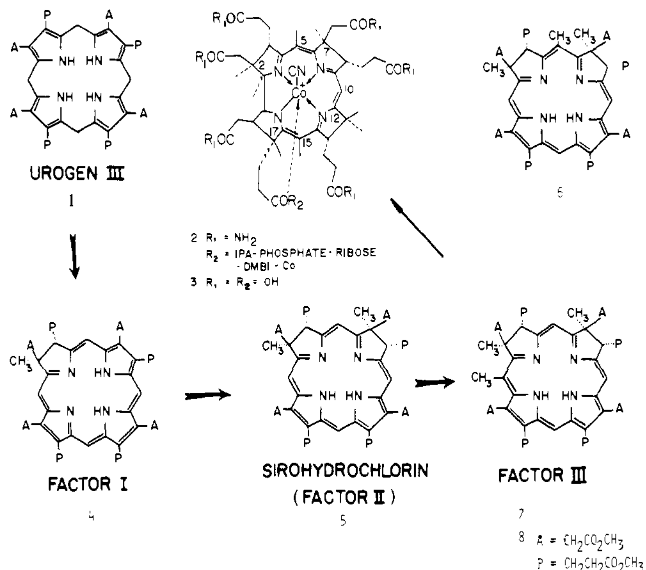
20-Methylsirohydrochlorin. A Revised Structure for a Trimethylisobacteriochlorin Intermediate (Factor III) in the Biosynthesis of Vitamin B_{12}

Sir:

With the establishment of the intermediacy of uro'gen III (**1**) in corrin biosynthesis,[1,2] a search for partially methylated metabolites as potential precursors of vitamin B_{12} (**2**) has so far led to the isolation and characterization[3,4] of three isobacteriochlorins from *Propionibacterium shermanii* and *Clostridium tetanomorphum* which undergo bioconversion to cobyrinic acid (**3**) and have been designated factors I–III (**4–6**).

The importance of these substances stems from their sequential position in post-uro'gen III metabolism and from the proven correspondence of factor II with sirohydrochlorin (**5**),[5-8] the iron-free prosthetic group of the sulfite and nitrite reductase enzymes[9] (siroheme). The proven[5] structural and stereochemical features of **5** led to the working structure **4** for factor I.[10] On the basis of ^{1}H NMR data for the bislactone of factor III (= corriphyrin-3),[6] structure **6** was proposed for the latter.[7,11] We now provide spectroscopic and biochemical evidence which requires that the "extra" methyl group is added



to sirohydrochlorin (**5**) at C-20 rather than at C-5, leading to the revised structure (**7**) for factor III, i.e., 20-methylsirohydrochlorin, which suffers a remarkable loss of C-20 with its attached methyl group on the way to vitamin B_{12}.

Factor III was isolated from δ-aminolevulinic acid (ALA) supplemented cobalt-free incubations of *P. shermanii* (ATCC 9614) and from a B_{12}-deficient mutant[12] of this organism. High resolution FD mass spectrometry of the octamethyl ester (**8**) established the formula $C_{51}H_{64}N_4O_{16}$ (988.4281), while the mass spectrum of the octa ester isolated from incubation with [*methyl*-$^2H_3$]-L-methionine revealed peaks at *m/e* 997, 994, 991, and 988 corresponding to enrichment with a maximum of three (M + 9) CD_3 groups. Analysis of the ^{1}H NMR spectrum (300 MHz) revealed only three signals at δ 6.43, 7.21, and 8.33 ppm in contrast to the four signals in this region in the spectrum of **5** which have been assigned to the four meso protons at C-5, C-10/C-20, and C-15 (δ 6.78, 7.36/7.46, and 8.54, respectively).[5] Factor III is therefore 10- or 20-methylsirohydrochlorin. In order to decide between these alternatives, a specimen of factor III (400 μg) was prepared from a suspended-cell incubation[5] of *P. shermanii* containing [$^{13}CH_3$]methionine and [5-^{13}C]-δ-aminolevulinic acid. When this ^{13}C-enriched species (as the octamethyl ester) was examined by ^{13}C NMR spectroscopy (Figure 1), it became

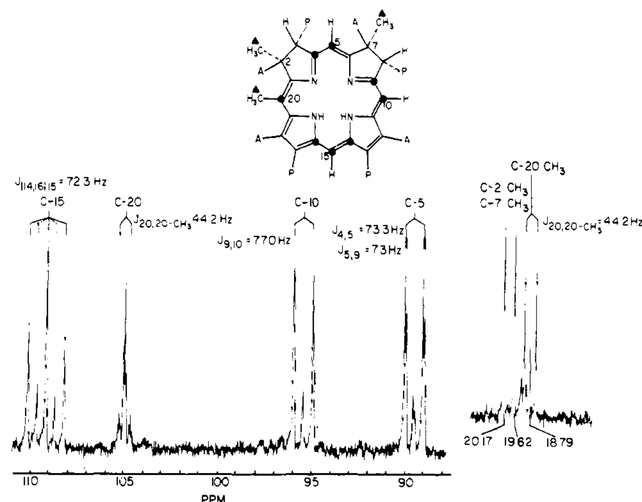


Figure 1. The meso carbon resonances of the proton noise decoupled ^{13}C FT spectrum of [5-^{13}C]-ALA- and [$^{13}CH_3$]methionine-labeled 20-methylsirohydrochlorin octamethyl ester. Inset: the methyl carbon resonances of the same sample; the spectrum was obtained in C_6D_6 at 75 MHz.

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