Manipulation of Vinyl Groups in Protoporphyrin IX: Introduction of Deuterium and Carbon-13 Labels for Spectroscopic Studies

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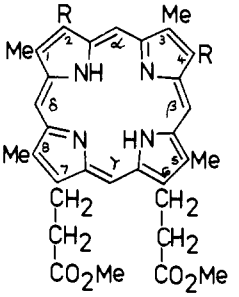
Abstract: Using commercially available hemin (**5**) as the starting material, routes for preparation of monovinyl deuterated (**27**), monovinyl carbon-13 enriched (**41**, **42**), and divinyl carbon-13 enriched (**43**, **44**) derivatives of protoporphyrin IX dimethyl ester (**1**) are described. The monovinyl carbon-13 enriched porphyrins **41** and **42** were obtained by way of a previously reported Wittig reaction on *Spirographis* and iso-*Spirographis* porphyrin dimethyl esters **28** and **39**, respectively. A new efficient partial synthesis of *Spirographis* porphyrin dimethyl ester (**28**) from deuteroporphyrin IX dimethyl ester (**7**) is reported, and in this the key formyl group at the 2 position is inserted by way of a Vilsmeier reaction employing a hindered amide.

Introduction

Proton NMR spectroscopy has been a particularly important technique for studying the electronic structure of paramagnetic porphyrins and heme proteins.[1,2] It is to be expected that carbon-13 and deuterium NMR will also contribute significantly to our understanding of the properties of diamagnetic hemes and heme proteins, as well as their paramagnetic counterparts. A significant series of advances in interpretation of the large isotropic NMR shifts[3-16] in hemes and heme proteins has been made possible by the availability of regioselectively deuterium-labeled hemes,[17-21] and in many cases order has been established where a great deal of confusion had existed. More recently, the interpretation of resonance Raman spectra of nickel porphyrins,[22] hemes,[23] and heme proteins[23,24] has been aided by the use of vinyl-labeled derivatives of protoporphyrin IX dimethyl ester (**1**).

We report here several methods which have been developed for the regiospecific incorporation of deuterium and carbon-13 labels into the α and β positions of the individual 2- and/or 4-vinyl groups in protoporphyrin IX dimethyl ester. This work introduces an added refinement to the pairwise (2 *and* 4 position) labeling of vinyl groups with deuterium[7] or with carbon-13[25] which have already been described.

Deuterium Labeling of Vinyl Groups in 1. In our earlier work[7] we successfully accomplished selective deuteration of the 2- *and* 4-α-vinyl and 2- *and* 4-β-vinyl protons in protoporphyrin IX dimethyl ester (**1**) by carrying out reactions on 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**2**). The vinyl-labeled



1 R = CH=CH$_2$
2 R = CO.Me
7 R = H
40 R = CHO
43 R = CH=$^{\bullet}$CH$_2$
44 R = $^{\bullet}$CH=CH$_2$
47 R = $^{\bullet}$CO.Me
48 R = $^{\bullet}$CH(OH).Me

$^{\bullet}$ indicates carbon-13 enrichment.

compounds enabled us to unequivocally identify the pairs of vinyls in proton NMR and resonance Raman spectra, but it was not possible to uniquely establish which vinyl was which when nonequivalence was unexpectedly identified using these spectroscopic techniques.[26] For this purpose we required a protoporphyrin IX

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derivative with only one of the 2,4-divinyl pair of substituents selectively labeled with deuterium, and subsequently with carbon-13 (see later). By analogy with our previous experience in the divinyl series, it seemed that an approach through selective manipulations of the 2- or 4-substituents in monoacetyldeuteroporphyrin IX derivatives held out the best hope for success in the proposed vinyl differentiation.

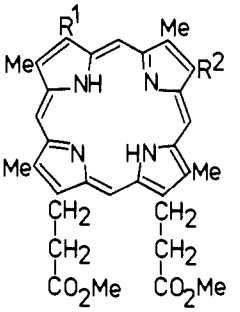
The initial phase of the project required synthesis and separation of large quantities of the monoacetyldeuteroporphyrin IX dimethyl ester isomers **3** and **4**. Commercially available hemin (**5**) was heated in a melt of resorcinol[27,28] and, following filtration, the crude deuterohemin (**6**) was subjected to demetalation using the Grinstein procedure[29] ($FeSO_4$/HCl/MeOH). Deuteroporphyrin IX dimethyl ester (**7**) was obtained in this way in 76% yield from **5**.

Although Friedel–Crafts acetylation proceeds well on the iron complex of **7**, pilot studies showed that the reaction was cleaner if the copper(II) complex **8** was used instead.[28] Acetylation of **8** using acetic anhydride and stannic chloride in dichloromethane gave a mixture containing the copper(II) complexes **9** and **10**/**11**, respectively, of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**2**) and the monoacetyl isomers **3**/**4**. These copper(II) complexes were readily separated by column chromatography (unlike the corresponding iron(III) complexes which require prior demetalation), and the copper was removed subsequently by stirring the metalloporphyrins in 10% sulfuric acid in trifluoroacetic acid. After further chromatography, the products (diacetyl **2** and monoacetyls **3**/**4**) were obtained in 67 and 24% yield, respectively, from **8**. A minor modification of this procedure, in which the copper(II) deuteroporphyrin IX dimethyl ester (**8**) was treated at 0 °C for only 20 s gave a separable mixture in which copper(II) monoacetyl isomers **10** and **11** predominated (60% yield, from **8**).

The method of separation for the monoacetyl isomers which was initially employed was thick-layer chromatography on silica gel G.[30] However, this approach was limited by the small amounts of porphyrin (40 mg) which could be applied to each 20 cm × 40 cm × 1 mm (thick) plate. Since gram quantities of the isomerically pure isomers **3** and **4** were expected to be required for development of the route to mono-vinyl-labeled protoporphyrin IX derivatives, medium-pressure (100–150 psi) liquid chromatography (MPLC) was used. Quantities of between 1 and 2 g of the mixed monoacetyl isomers **10** and **11** could be applied to the Whatman LPS-1 column, and about 80% of the eluted porphyrin was collected as isomerically pure product. The copper(II) 2-acetyldeuteroporphyrin IX dimethyl ester (**10**) was the more mobile iosmer and was generally found in greater abundance than the 4-acetyl isomer **11**. Elution of the isomers from the MPLC system usually required about 20 h, and the copper(II) complexes of the porphyrins were used because these seemed to suffer less degradation in that time than the corresponding metal-free porphyrins. Demetalation, as previously, gave the individual pure monoacetyl isomers **3** and **4** from their copper(II) complexes **10** and **11**.

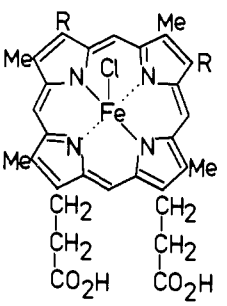
Initial plans to accomplish regioselective vinyl labeling revolved around protection of the acetyl function on an iron or copper monoacetyldeuteroporphyrin IX dimethyl ester as the corresponding dithioketal,[32] followed by Friedel–Crafts acetylation at the free position, reduction, and dehydration to give vinyl at this position, and then deprotection and labeling of the regenerated acetyl, as previously described,[7] to give either α- or β-labeled vinyl. This approach was unexpectedly thwarted by deacetylation of the copper(II) porphyrin under the conditions employed to form the dithioketal;[33] though the dithioketal of the metal-free porphyrin

Chart I

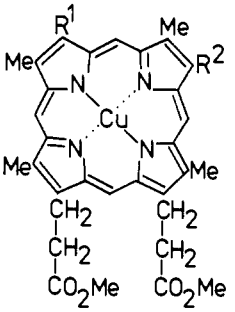


3 R^1 = CO.Me; R^2 = H
12 R^1 = CH(OH).Me; R^2 = H
14 R^1 = $CH_2CH(OMe)_2$; R^2 = H
16 R^1 = CH_2CH_2OH; R^2 = H
18 R^1 = CH_2CH_2Cl; R^2 = H
20 R^1 = CH_2CH_2Cl; R^2 = $CO.CD_3$
22 R^1 = CH_2CH_2D; R^2 = $CD{=}CD_2$
25 R^1 = CH_2CH_2OH; R^2 = $CO.CD_3$
27 R^1 = $CH{=}CH_2$; R^2 = $CD{=}CD_2$
29 R^1 = H; R^2 = CH(OH).Me
31 R^1 = H; R^2 = CH_2CH_2OH
35 R^1 = CHO; R^2 = CH_2CH_2Cl
38 R^1 =  ; R^2 = $CH{=}CH_2$
41 R^1 = $CH{=}\overset{\bullet}{C}H_2$; R^2 = $CH{=}CH_2$
45 R^1 = $\overset{\bullet}{C}O.Me$; R^2 = H

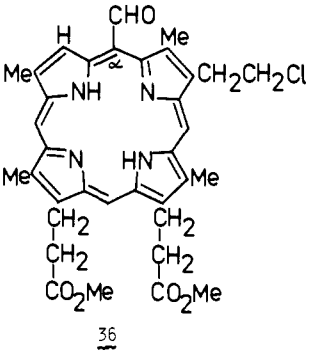
4 R^1 = H; R^2 = CO.Me
13 R^1 = $CH{=}CH_2$; R^2 = H
15 R^1 = CH_2CHO; R^2 = H
17 R^1 = CH_2CH_2OAc; R^2 = H
19 R^1 = CH_2CH_2Cl; R^2 = CO.Me
21 R^1 = CH_2CH_2Cl; R^2 = $CD{=}CD_2$
24 R^1 = CH_2CH_2OAc; R^2 = CO.Me
26 R^1 = CH_2CH_2OH; R^2 = $CD(OH).CD_3$
28 R^1 = CHO; R^2 = $CH{=}CH_2$
30 R^1 = H; R^2 = $CH{=}CH_2$
33 R^1 = H; R^2 = CH_2CH_2Cl
37 R^1 =  ; R^2 = CH_2CH_2Cl
39 R^1 = $CH{=}CH_2$; R^2 = CHO
42 R^1 = $CH{=}CH_2$; R^2 = $CH{=}\overset{\bullet}{C}H_2$
46 R^1 = H; R^2 = $\overset{\bullet}{C}O.Me$



5 R = $CH{=}CH_2$
6 R = H



8 R^1 = R^2 = H
9 R^1 = R^2 = CO.Me
10 R^1 = CO.Me; R^2 = H
11 R^1 = H; R^2 = CO.Me
23 R^1 = CH_2CH_2OAc; R^2 = H
32 R^1 = H; R^2 = CH_2CH_2OH
34 R^1 = CHO; R^2 = CH_2CH_2Cl



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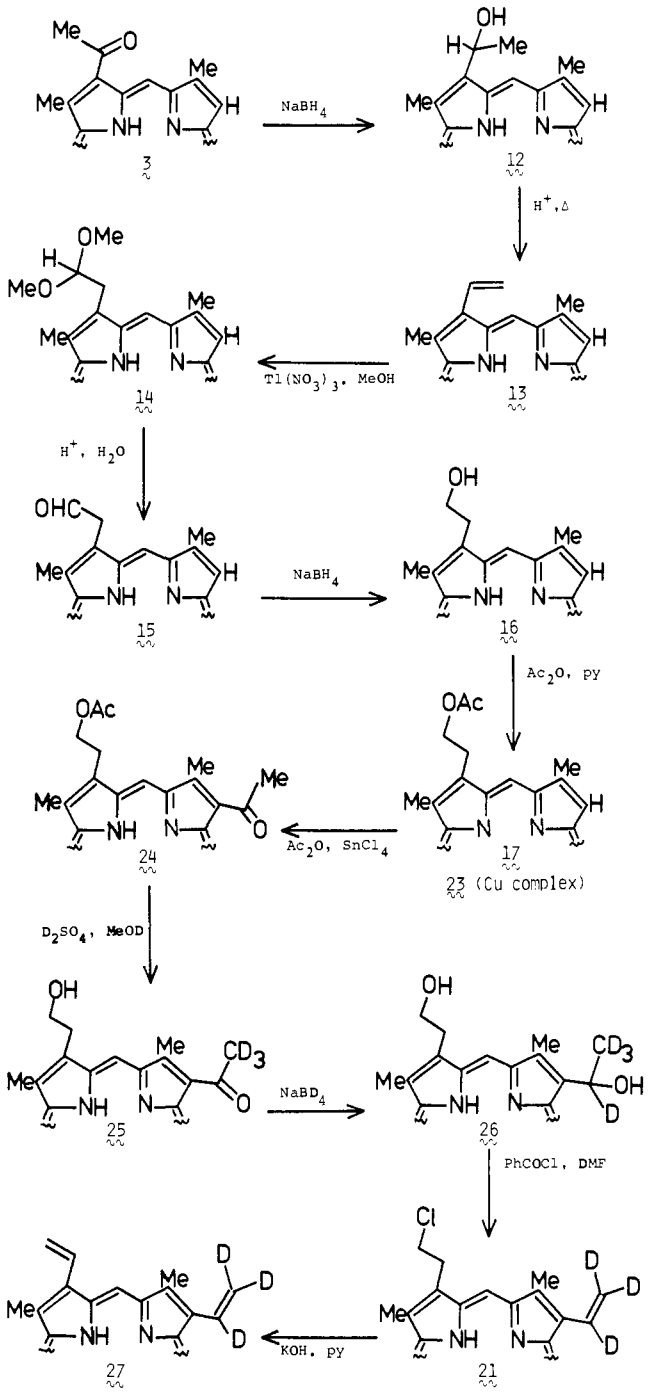
(26) NMR[9] and resonance Raman[23] data indicate that one vinyl group in myoglobin is significantly more mobile than the other, and NMR studies[8] have revealed a pH dependence of the oscillatory mobility of vinyl groups in proteins, suggesting that the vinyl hydrogen assignments could be very important for NMR and resonance Raman studies of hemoglobin cooperativity.
(27) Fuhrhop, J.-H.; Smith, K. M. In "Porphyrins and Metalloporphyrins"; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; p 822.
(28) Brockmann, H., Jr.; Bliesener, K.-M.; Inhoffen, H. H. *Justus Liebigs Ann. Chem.* **1968**, *718*, 148–161.
(29) Reference 27, pp 802–803.
(30) Fractional crystallization[31] of the mixture was erratic in our hands.
(31) Miller, M. J.; Rapoport, H. *J. Am. Chem. Soc.* **1977**, *99*, 3479–3485.
(32) Deprotection would probably have occurred during the Friedel–Crafts acylation and workup if the simple ethylene glycol ketal had been employed; we therefore chose a more robust protecting group.

Scheme I



could be formed, insertion of copper and subsequent Friedel–Crafts acetylation also accomplished deacetylation of the protected acetyl group.

It was therefore decided to protect the two-carbon side chain on the monosubstituted deuteroporphyrin IX in the form of the corresponding 2-chloroethyl; this would not involve a great increase in the number of synthetic steps, and vinyls can be efficiently regenerated from 2-chloroethyls by treatment with base.[17,34] Vinylporphyrins themselves cannot be subjected to Vilsmeier or Friedel–Crafts reactions owing to unwanted side reactions.[35]

As shown in Scheme I, 2-acetyldeuteroporphyrin IX dimethyl ester (**3**) was reduced with sodium borohydride to give the 2-(1-hydroxyethyl) derivative **12**; acid-catalyzed dehydration in *o*-dichlorobenzene[36] gave 2-vinyldeuteroporphyrin IX dimethyl ester (isopemptoporphyrin dimethyl ester, **13**) in 83% overall yield from **3**. Vinyl protection was accomplished using the thallium trinitrate route;[37] with 2.2 equiv of thallium(II) trinitrate in presence of methanol, compound **13** gave the corresponding dimethyl acetal **14** after removal of chelated thallium(III). The acetal was hydrolyzed, and the resulting aldehyde, **15**, was reduced with sodium borohydride to give 2-(2-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**16**) in 87% yield from 13. At this point the 2-(2-hydroxyethyl) group was protected as the corresponding acetate **17**; however, prior to this, in an unsuccessful alternate route, the 2-(2-hydroxyethyl)porphyrin **16** was converted into the 2-(2-chloroethyl) derivative **18**, subjected to Friedel–Crafts acetylation (acetic anhydride and stannic chloride) to give **19**, and then deuterated (D_2SO_4/MeOD) to give **20**. Sodium borodeuteride reduction, followed by acid-catalyzed dehydration, gave an inseparable mixture of products which was shown by ^1H NMR analysis to consist of the required vinylporphyrin **21** and the analogous 2-(2-deuterioethyl)-4-vinylporphyrin **22**; the latter compound was obviously produced from **20** by deuteride displacement of the 2-chloro group, and thereby demonstrated that chloroethyl protection of a vinyl group was unsatisfactory in this particular sequence. It was for this reason that we reverted to the 2-(2-acetoxyethyl) protection existing in **17**.

Treatment of porphyrin **17** with copper(II) acetate in methanol/dichloromethane gave the copper complex **23** which was subjected to Friedel–Crafts acetylation to give, after removal of the copper (H_2SO_4/trifluoroacetic acid), a 64% yield of 2-(2-acetoxyethyl)-4-acetyldeuteroporphyrin IX dimethyl ester (**24**). This was dissolved in D_2SO_4/MeOD and underwent acetyl deuteration with concomitant methanolysis of the 2-acetate function to give the 2-(2-hydroxyethyl)-4-acetylporphyrin **25**.[38] Sodium borodeuteride reduction gave the 2-(2-hydroxyethyl)-4-(1-hydroxyethyl)porphyrin **26**, which, upon treatment with benzoyl chloride and dimethylformamide[39] gave a 77% yield of the 2-(2-chloroethyl)-4-vinylporphyrin **21**. With sodium hydroxide in pyridine,[34] an 83% yield of the required porphyrin, **27**, was obtained.

Repetition of the above sequence of reactions, but using the 4-acetyl isoomer, **4**, would afford the isomeric 2-vinyl-labeled protoporphyrin IX derivative, but for the purposes of the proposed studies, compound **27** alone whould be sufficient to definitively differentiate between vinyls 2 and 4 in heme proteins reconstituted with the heme derived from it. Instead, the 4-acetyl material, **4**, was used to develop a new partial synthesis of chlorocruoroporphyrin which could be used for carbon-13 enrichment of the 2-β-vinyl carbon in protoporphyrin IX.

Carbon-13 Labeling of Vinyl Groups in 1. Partial Synthesis of Chlorocruoroporphyrin (*Spirographis* porphyrin). Several total[40] and partial[41] syntheses of chlorocruoroporphyrin have already been reported. Our development of a new partial synthesis of chlorocruoroporphyrin (*Spirographis* porphyrin) dimethyl ester (**28**) was prompted by the anticipated need (see later) for a formyl–vinyl porphyrin which could be transformed into a specifically labeled 2,4-divinylporphyrin by way of a Wittig reaction using a carbon-13 enriched ylide. We were also interested in demonstrating the

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(34) Clezy, P. S.; Fookes, C. J. R. *Aust. J. Chem.* **1977**, *30*, 217–220.
(35) E. g., Nichol, A. W. *J. Chem. Soc. C* **1970**, 903–910.
(36) Reference 27, p 771.
(37) Kenner, G. W.; McCombie, S. W.; Smith, K. M. *Justus Liebigs Ann. Chem.* **1973**, 1329–1338.
(38) TLC, NMR, and spectrophotometry showed that the major product from this reaction was the corresponding dimethyl ketal of the required acetylporphyrin; this necessitated the inclusion of an extra hydrolysis step.
(39) Clezy, P. S.; Fookes, C. J. R.; Sternhell, S. *Aust. J. Chem.* **1978**, *31*, 639–648.
(40) (a) Jackson, A. H.; Kenner, G. W.; Wass, J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 480–490. (b) Bamfield, P.; Grigg, R.; Johnson, A. W.; Kenyon, R. W. *J. Chem. Soc. C* **1968**, 1259–1265. (c) Clezy, P. S.; Diakiw, V. *Aust. J. Chem.* **1975**, *28*, 2703–2725.
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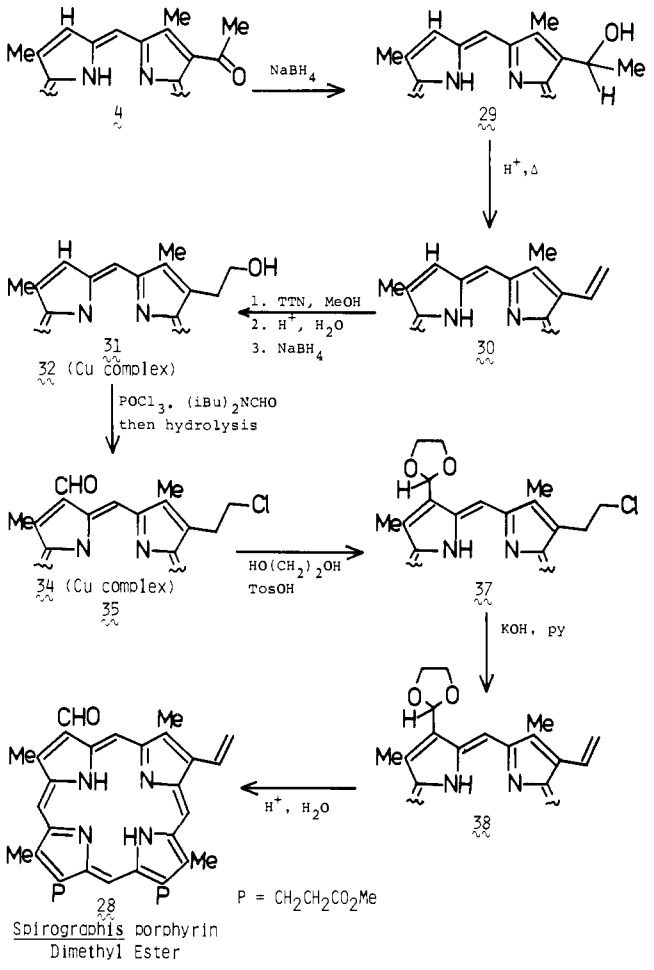
Scheme II



synthetic utility of our recently reported[42] method for formylation at the β positions in porphyrins using a hindered Vilsmeier reagent. Moreover, we were in possession of considerable quantities of 4-acetyldeuteroporphyrin IX dimethyl ester (**4**) which could be used for these purposes. The successful route is shown in Scheme II.

4-Acetyldeuteroporphyrin IX dimethyl ester (**4**) was reduced with sodium borohydride, and the resulting 4-(1-hydroxyethyl)-porphyrin, **29**, was dehydrated using *p*-toluenesulfonic acid in *o*-dichlorobenzene, to give a 75% yield (from **4**) of pempto-porphyrin dimethyl ester (**30**). Application of the thallium(III) trinitrate route (see above) gave an 86% yield of 4-(2-hydroxy-ethyl)deuteroporphyrin IX dimethyl ester (**31**) in three steps. The copper(II) complex **32**, obtained from **31**, was treated with 60 equiv of the preformed phosphoryl chloride/diisobutylformamide complex[42] in 1,2-dichloroethane. While being heated at 70 °C the reaction mixture was monitored by analytical TLC, and this showed the 2-hydroxyethyl group to be rapidly transformed into 2-chloroethyl, but imine salt formation was much slower. After 7 h, reaction progress seemed to have stopped, so the mixture was hydrolyzed (imine salt–aldehyde) and a thick-layer preparative plate separation afforded three major bands. The most mobile, least abundant band was the copper(II) 4-(2-chloroethyl)-porphyrin, which after demetalation gave a 16% yield of 4-(2-chloroethyl)deuteroporphyrin IX dimethyl ester (**33**). This band was followed by a much slower running green-colored fraction which was presumably a meso-formylated[42] 4-(2-chloroethyl)-porphyrin. Closely trailing this material was the required copper(II) 2-formyl-4-(2-chloroethyl)deuteroporphyrin IX dimethyl ester (**34**). After demetalation (H_2SO_4/trifluoroacetic acid), the 2-formyl-4-chloroethylporphyrin, **35**, was obtained in 44% yield (or 52% based on recovered **33**). NMR spectroscopy of the meso-formylated fraction indicated only one isomer to be present, this probably being the *α-meso*-formyl-4-(2-chloroethyl)-deuteroporphyrin IX dimethyl ester (**36**).[28,42] Treatment of **35** with ethylene glycol and *p*-toluenesulfonic acid gave the acetal **37**, which was then dehydrochlorinated with potassium hydroxide in pyridine/methanol to give the vinyl acetal **38**. Finally, regeneration of the formyl group by treatment with aqueous acid gave *Spirographis* porphyrin dimethyl ester (**28**) in 75% overall yield from **35**. This material was identical, by melting point and standard spectroscopic techniques, with an authentic sample.

Wittig Reactions on Chlorocruoroporphyrin and Its Isomer. Syntheses of Mono-β-vinyl Carbon-13 Enriched Derivatives of Protoporphyrin IX Dimethyl Ester. The chlorocruoroporphyrin synthesis described above proved to be too lengthy to apply in large-scale syntheses of **28** and its isomer, **39**. These two compounds were therefore synthesized from protoporphyrin IX dimethyl ester (**1**) using osmium tetroxide/periodate[41c,43] or by careful ozonolysis,[44] which afforded a mixture consisting of starting material, **1**, the monoformylmonovinylporphyrins **28** and **39**, and the corresponding 2,4-diformyldeuteroporphyrin IX dimethyl ester (**40**). These compounds were separated initially into three fractions (**1**, **28**/**39**, **40**) by column chromatography on alumina, and the monoformyl mixture was then separated using MPLC on Whatman LPS-1 silica; in this final separation, the 2-formyl-4-vinyl isomer **28** was eluted before **39**. Identification of the individual isomers was made by melting point, mixture melting point, and finally 360-MHz NMR spectroscopy.

The zinc(II) complexes of compounds **28**, **39**, and **40** were treated with 99% enriched [^{13}C]methyltriphenylphosphonium iodide in the presence of butyllithium, and afforded 40 to 50% yields of the corresponding vinyl-labeled protoporphyrins IX, **41**, **42**, and **43**, respectively, after removal of the chelating zinc. These samples were identical with authentic protoporphyrin IX dimethyl ester (**1**), except that in the proton NMR spectrum the vinyl CH_2 resonances experienced a 156–159-Hz ($J_{C\text{-}13\text{-}H}$) coupling in labeled vinyls. In the carbon-13 NMR spectrum an enhanced resonance at ca. 120.9 ppm was observed which, in the proton-coupled mode, was observed as a triplet with $J_{C\text{-}13\text{-}H} \sim 158$ Hz.

Synthesis of Protoporphyrin IX Dimethyl Ester with Carbon-13 Labels at the α-Vinyl Positions. The most logical approach to this porphyrin, **44**, appeared to be via Friedel–Crafts acetylation using 99% 1-^{13}C enriched acetic acid. On the small scales used, it was most convenient to transform commercially available 1-^{13}C enriched sodium acetate into acetyl chloride, rather than into acetic anhydride. The acetate was therefore treated with oxalyl chloride and afforded acetyl chloride, 1-^{13}C enriched, in good yield.[45]

In trial reactions with unlabeled reagents, pure deuterohemin dimethyl ester (**6**) (obtained by reinsertion of iron into pure deuteroporphyrin IX dimethyl ester, rather than directly from the resorcinol fusion reaction) reacted very much slower with acetyl chloride than with acetic anhydride in the presence of stannic chloride. The major products always appeared to be the iron(III) complexes of the monacetylated species **3** and **4**; the best conditions appeared to involve treatment of deuterohemin dimethyl ester with acetyl chloride and stannic chloride at 0 °C for 12 h, under nitrogen. After a workup in which the iron was removed by the Grinstein procedure,[29] column chromatography afforded three bands which were shown to be, in order of increasing polarity, (1) deuteroporphyrin IX dimethyl ester (**7**) (30% recovery), (2) mixed ^{13}C-labeled monoacetyldeuteroporphyrin IX dimethyl esters (**45** and **46**), and (3) double labeled 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**47**). Identities of these compounds were established by melting point comparisons and NMR spectroscopy. The presence of carbon-13 was established by carbon-13 NMR spectroscopy; for example, compound **47** showed one enriched

(42) Smith, K. M.; Langry, K. C. *J. Chem. Soc., Perkin Trans. 1* **1983**, 439–444.

(43) Sparatore, F.; Mauzerall, D. *J. Org. Chem.* **1960**, *25*, 1073–1076.
(44) Fuhrhop, J.-H., personal communication.
(45) Kenner, G. W.; Rimmer, J.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc., Perkin Trans. 1* **1977**, 332–340.

signal for both labeled carbons around 200 ppm which did not change its multiplicity when run with proton coupling. The 2,4-diacetyldeuteroporphyrin **47** was treated with sodium borohydride to afford the corresponding hematoporphyrin IX, **48**, which was dehydrated, either with *p*-toluenesulfonic acid in *o*-dichlorobenzene[36] or with benzoyl chloride and dimethylformamide,[39] to give the doubly labeled protoporphyrin IX dimethyl ester derivative **44**. Compound **4**, showed, once again, a single carbon-13 resonance for both side chains at about 62.5 ppm, and in the proton-coupled spectrum this split into two peaks (J_{C-H} = 140.6 Hz). In the carbon-13 NMR spectrum of the final product, **44**, one enriched peak appeared at about 130 ppm, and this split into a doublet (J_{C-H} = 153.4 Hz) in the proton-coupled spectrum.

Insertion of Iron into the Labeled Porphyrins. Many methods for insertion of iron into porphyrins exist,[46] but few are suitable for use with sensitive porphyrin and chlorophyll systems. The most widely used method[47] employs treatment of the porphyrin in a small amount of pyridine with ferrous sulfate in hot acetic acid. In our hands this method can result in decomposition (a base-line spot is almost always observed during TLC analysis) and loss of deuterium labels. Thus, to avoid these losses a new method was developed; this involves addition of a solution of porphyrin in nitrogen-purged chloroform to deoxygenated acetonitrile containing ferrous chloride hydrate.[48] The method usually calls for an equal weight of ferrous chloride to porphyrin, but there is no doubt that this convenience can be adjusted to use much smaller amounts of the chelating metal. For porphyrins with relatively stable side chains (deuteroporphyrin, protoporphyrin, etc.), the reaction can be performed at 50–60 °C and requires only 15 min. With more sensitive porphyrins, such as deuterium-labeled ones, or hematoporphyrin IX (which suffers dehydration using the usual method[47]), the reaction is conducted at lower temperatures, and takes correspondingly longer. After completion of the reaction (monitored by analytical TLC), the mixture is exposed to oxygen and worked up in the usual way. Carrying out the reaction in acetonitrile usually results in the ferrous chloride being partially in suspension, but acetonitrile can be substituted with methanol as a solvent if this should prove to be troublesome; however, although ferrous sulfate is considerably more soluble in methanol than in acetonitrile, the opposite is true for the porphyrin. Thus, a change to methanol may cause the precipitation of the porphyrin, and since we regard this as a much more serious problem, we prefer to use acetonitrile as the solvent.

Experimental Section

Melting points were measured on a hot-stage apparatus and are uncorrected. Neutral alumina (Merck) was used for column chromatography, and preparative TLC was carried out on 20 × 20 cm glass plates coated with Merck GF 254 silica gel (1 mm thick). Analytical TLC was performed using Merck silica gel 60 F 254 precoated sheets (0.2 mm). Medium-pressure liquid chromatography (MPLC) was carried out using a 2.5 × 100 cm (300 psi rated) glass column (Altex), packed with Whatman silica (LPS-1; 13–24 μ); compounds were eluted at 100–150 psi with a $^1/_4$ in. piston-driven FMI pump, and an ISCO 1840 variable-wavelength detector (set at 405 nm) was used. High-pressure liquid chromatography (HPLC) was performed on a Waters Associates chromatograph, with a Model 6000A solvent delivery system, a U6K injector, a Perkin-Elmer LC 55B variable-wavelength detector (set at 405 nm), and a microporasil (250 × 4.6 mm i.d.) column eluted with tetrahydrofuran or methanol in dichloromethane. Electronic absorption spectra were measured on a Cary 17 spectrophotometer (solutions in dichloromethane), proton NMR spectra were measured at 360 MHz using a Nicolet NT-360 spectrometer, and carbon-13 NMR spectra were measured using a Nicolet NT-200 instrument (solutions in $CDCl_3$). Mass spectra were measured (direct insertion probe, 70 eV, 50 μA, source temp. ca. 200 °C) using a Finnegan 3200 mass spectrometer.

Hemin chloride (**5**) was purchased from Man-Win (Washington, D.C.). Elemental analyses were performed at the Berkeley Microanalytical Laboratory, Department of Chemistry, University of California—Berkeley.

2-Acetyl-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (3) and 4-Acetyl-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (4). To 1.92 g of copper(II) deuteroporphyrin IX dimethyl ester in 150 mL of dichloromethane was added 40 mL of acetic anhydride. The mixture was cooled to 0 °C, which caused a substantial amount of the porphyrin–copper complex to precipitate from solution. Freshly distilled stannic chloride (4 mL) was added, and the resulting homogeneous green solution was stirred for 20 s before being poured over 200 g of ice. The mixture was diluted with 200 mL of dichloromethane, and the organic solvent was evaporated under vacuum to give a residue which was chromatographed on neutral alumina (Brockmann Grade III, elution with dichloromethane initially, and then with 0.5% methanol in dichloromethane, to remove diacetylated material). The separated fractions were crystallized from dichloromethane/heptane to give the mixed copper(II) 2- and 4-monoacetyldeuteroporphyrin IX dimethyl esters (**10** and **11**) in a combined yield of 60% (804 mg). The copper(II) complex of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**2**) was obtained in 27% yield (364 mg).

Separation of the Isomers 10 and 11. Small quantities of the copper(II) monoacetyl isomers (ca. 30–50 mg per plate) could be separated on 20 × 40 cm × 1 mm thick silica gel G preparative TLC plates (elution with 2% tetrahydrofuran in dichloromethane). More convenient large-scale (1–2 g) separations of these compounds **10** and **11** was achieved with medium-pressure (100–150 psi) liquid chromatography (MPLC). With the aid of an infusion pump, ca. 1.5 g of the isomer mixture, dissolved in 30 mL of dichloromethane and contained in a 35-mL plastic syringe, was transferred at a rate of 2.5 mL/min onto a column (2.5 cm × 100 cm) of silica gel (Whatman LPS-1) which had previously been equilibrated with 2% tetrahydrofuran in dichloromethane. Once the solution had been applied to the column, the compounds were eluted with 2% tetrahydrofuran in dichloromethane, a process which usually required 6–10 h before the first fraction began to exit the system. An additional 3–6 h was necessary for the compounds to completely pass through the column; the effluent was collected in 15-mL aliquots using a Gilson microfractionator. Since TLC analysis of the fractions was relatively insensitive, HPLC analysis was used (microporasil column (25 cm × 0.4 cm), eluted with 1% tetrahydrofuran in dichloromethane) to assay the fractions for purity.

Demetalation. To the metalloporphyrins in 30 mL of trifluoroacetic acid was added 3.0 mL of concentrated sulfuric acid, and the mixture was stirred for 1 h at room temperature. The mixtures were then each diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water; the organic phases were then treated briefly with excess ethereal diazomethane. After evaporation of the solvent, the residues were filtered through a short plug of neutral alumina (Brockmann Grade III, elution with dichloromethane) to give the purified monoacetyl porphyrin free bases. A comparison of the melting point data identified the more chromatographically mobile copper complex as that of the 2-acetyl isomer **10**. In a typical separation, 1.538 g of the mixed copper(II) complexes **10** and **11** gave, after MPLC and demetalation, 0.658 g (47% yield) of the 2-acetylporphyrin **3** and 0.608 g (44% yield) of the 4-acetyldeuteroporphyrin **4**, for a total recovery of 91% of the purified isomers.

Most Mobile Fraction: 2-Acetyldeuteroporphyrin IX Dimethyl Ester (3): mp 238–239 °C (lit.[31] mp 238.5–240.5 °C); MS, *m/e* (%), 580 (100), 565 (4), 549 (4), 521 (6), 507 (39), 447 (8), 434 (11); ^{1}H NMR δ (ppm) 3.29 (m, 4 H, CH_2CO), 3.34 (s, 3 H, COMe), 3.56, 3.72, 3.80, 3.93 (each s, 3 H, Me), 3.65, 3.68 (each s, 3 H, OMe), 4.34, 4.48 (each t, 2 H, CH_2CH_2CO), 9.14 (s, 1 H, 4-H), 9.98, 10.03, 10.21, 10.86 (each s, 1 H, meso H).

Least Mobile Fraction: 4-Acetyldeuteroporphyrin IX Dimethyl Ester (4): mp 214–216 °C (lit.[31] mp 211–213 °C); MS, *m/e* (%), 580 (100), 565 (4), 549 (5), 521 (6), 507 (39), 447 (9), 434 (11); ^{1}H NMR δ (ppm) 3.28 (q, 4 H, CH_2CO), 3.34 (s, 3 H, COMe), 3.58, 3.72, 3.79, 3.91 (each s, 3 H, Me), 3.66, 3.68 (each s, 3 H, OMe), 4.35, 4.48 (each t, 2 H, CH_2CH_2CO), 9.19 (s, 1 H, 2-H), 10.05, 10.16, 10.83 (each s, 2 H, 1 H, 1 H, meso H).

6,7-Bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-vinylporphyrin, "Isopemptoporphyrin Dimethyl Ester" (13). To 71 mg of 2-acetyldeuteroporphyrin IX dimethyl ester (**3**) dissolved in 40 mL of dichloromethane was added 10 mL of an ice-chilled solution of methanol containing 175 mg of sodium borohydride. The mixture was stirred for 10 min at room temperature, when it was determined by TLC that reduction of the acetyl group was complete. Acetic acid (3 mL) was added to quench excess borohydride, and the solution was diluted with 50 mL of dichloromethane and then washed with 2 × 100 mL of water. The

(46) Buchler, J. W. In ref 27, Chapter 5.
(47) Fuhrhop, J.-H.; Smith, K. M. In ref 27, p 803.
(48) Baldwin and co-workers have developed a similar method using tetrahydrofuran as the solvent: Almog, J.; Baldwin, J. E.; Huff, J. *J. Am. Chem. Soc.* **1975**, *97*, 227–228. Almog, J.; Baldwin, J. E.; Crossley, M. J.; Debernardis, J. F.; Dyer, R. L.; Huff, J. R.; Peters, M. K. *Tetrahedron* **1981**, *37*, 3589–3601.

organic fraction was collected and the solvent was evaporated under vacuum. The residue (containing porphyrin **12**) was dissolved in 40 mL of o-dichlorobenzene containing 180 mg of *p*-toluenesulfonic acid hydrate, and heated at 145 °C for 40 min as nitrogen gas was rapidly passed through the solution. The cooled reaction mixture was diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water. The vinylporphyrin solution was collected, the solvent evaporated, and the residue dissolved in 50 mL of dichloromethane before being treated with excess ethereal diazomethane. After evaporation of the solvent, the residue was chromatographed on 1 mm thick 20 × 20 cm preparative silica gel TLC plates (elution with 2% methanol in dichloromethane). The major band was collected and the porphyrin was recovered from the silica gel by washing with 3% methanol in dichloromethane. Evaporation of the solvent at reduced pressure, followed by crystallization of the purified porphyrin from dichloromethane/heptane, gave 57 mg (83% yield) of isopemptoporphyrin dimethyl ester, mp 219–221 °C (lit.[40c] mp 219–221 °C). A mixture melting point with authentic material showed no depression: MS, *m/e* (%), 564 (100), 491 (38), 417 (15); ^{1}H NMR δ (ppm) 3.27 (t, 4 H, CH_2CO), 3.59, 3.63, 3.70, 3.73 (each s, 3 H, Me), 3.64, 3.65 (each s, 3 H, OMe), 4.39 (dt, 4 H, CH_2CH_2CO), 6.17 (dd, 1 H, *cis* β-vinyl CH, J = 12 Hz), 6.36 (dd, 1 H, trans β-vinyl CH, J = 18 Hz), 8.28 (q, 1 H, α-vinyl CH), 9.06 (s, 1 H, 4-H), 9.98, 10.02, 10.07, 10.19 (each s, 1 H, meso H).

6,7-Bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-4-vinylporphyrin, "Pemptoporphyrin Dimethyl Ester" (30). This compound was similarly prepared from 4-acetyldeuteroporphyrin IX dimethyl ester (**4**) in 75% yield (73 mg) and had mp 211–213 °C (lit.[40a] mp 213–214 °C). A mixture melting point with an authentic sample showed no depression: MS, *m/e* (%), 564 (100), 533 (8), 505 (9), 491 (38), 431 (10); ^{1}H NMR δ (ppm) 3.32 (t, 4 H, CH_2CO), 3.67, 3.73, 3.77 (each s, 12 H, 3 H, 3 H, Me, and OMe), 4.46 (dt, 4 H, CH_2CH_2CO), 6.19 (dd, 1 H, cis β-vinyl CH, J = 12 Hz), 6.38 (dd, 1 H, trans β-vinyl CH, J = 18 Hz), 8.32 (q, 1 H, α-vinyl CH), 9.12 (s, 1 H, 2-H), 10.11, 10.14, 10.23 (each s, 2 H, 1 H, 1 H, meso H).

2-(2-Hydroxyethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (16). To a solution of 100 mL of dichloromethane and 15 mL of anhydrous methanol containing 510 mg of 2-vinyldeuteroporphyrin IX dimethyl ester (**13**) was added 884 mg (2.2 mol equiv) of thallium(III) trinitrate trihydrate dissolved in 25 mL of anhydrous methanol. The mixture was stirred for 12 min at 40 °C, and then, after cooling, sulfur dioxide gas was bubbled through the mixture for 1 min. Concentrated hydrochloric acid (1.5 mL) was added and the mixture was stirred for 2 min before the supernatant was decanted and the precipitated thallium(I) salts were washed with additional solvent. The combined organic solutions were washed three times with 100 mL of water and evaporated under vacuum; the residue (compound **14**) was dissolved in 100 mL of tetrahydrofuran containing 3 mL of water and 1.5 mL of concentrated hydrochloric acid. This solution was refluxed for 5 min, cooled briefly, diluted with 100 mL of dichloromethane, and then washed twice with water. The solvent was evaporated under vacuum and the residue was dissolved in an arbitrary mixture of tetrahydrofuran/methanol/dichloromethane. The solution of aldehyde **15** was treated at 0 °C with 800 mg of sodium borohydride in 30 mL of ice-cold methanol. It was stirred for 10 min before 3 mL of acetic acid was added to quench excess borohydride. The solvent was evaporated under vacuum and the residue was stirred for 12 h in 150 mL of dry methanol containing 8 mL of concentrated sulfuric acid. The acidic solution was then diluted with 150 mL of dichloromethane and washed with 2 × 150 mL of water. After chromatography on neutral alumina (Brockmann Grade III, elution with chloroform), the red eluates were evaporated and the residue was crystallized from dichloromethane/heptane to give 460 mg (87% yield) of **16**: mp 213–215 °C (lit.[40a] mp 210–212 °C); MS, *m/e* (%), 582 (100), 551 (50), 509 (37), 478 (13), 466 (9), 405 (21); ^{1}H NMR δ (ppm) 3.28 (t, 4 H, CH_2CO), 3.61, 3.65, 3.68, 3.74 (each s, 3 H, 9 H, 3 H, 3 H, Me, and OMe), 4.40 (m, 8 H, CH_2CH_2CO and CH_2CH_2O), 9.08 (s, 1 H, 4-H), 10.03, 10.10, 10.12 (each s, 1 H, 1 H, 2 H, meso H).

Anal. Calcd for $C_{34}H_{38}N_4O_5$: C, 70.08; H, 6.57; N, 9.62. Found: C, 70.03; H, 6.58; N, 9.58.

4-(2-Hydroxyethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin, (31). This compound was likewise prepared from 432 mg of 4-vinyldeuteroporphyrin IX dimethyl ester (**30**) in 86% yield (384 mg): mp 216–218 °C (lit.[40a] mp 215–217 °C); MS, *m/e* (%), 582 (100), 581 (65), 551 (30), 509 (28), 405 (15); ^{1}H NMR δ (ppm) 3.30 (t, 4 H, CH_2CO), 3.66, 3.75 (each s, 15 H, 3 H, Me, and OMe), 4.40 (m, 8 H, CH_2CH_2CO and CH_2CH_2O), 9.10 (s, 1 H, 2-H), 10.06, 10.10, 10.13, 10.15 (each s, 1 H, meso H).

Anal. Calcd for $C_{34}H_{38}N_4O_5$: C, 70.08; H, 6.57; N, 9.62. Found: C, 69.98; H, 6.69; N, 9.59.

2-(2-Acetoxyethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (17). To 310 mg of 2-(2-hydroxyethyl)deuteroporphyin IX dimethyl ester (**16**) in 40 mL of pyridine was added 5 mL of acetic anhydride. The reaction mixture was stirred at room temperture for 20 h and then diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water. The organic fraction was collected and evaporated to dryness; the residue was chromatographed on neutral alumina (Brockmann Grade III, elution with chloroform). The red eluates were evaporated and the residue was crystallized from dichloromethane/heptane to give 323 mg (97% yield) of the porphyrin acetate: mp 213–215 °C (lit.[40a] mp 213–215 °C); MS, *m/e* (%), 624 (100), 583 (3), 564 (12), 551 (48), 491 (20), 478 (16); ^{1}H NMR δ (ppm) 2.01 (s, 3 H, OCOMe), 3.29 (t, 4 H, CH_2CO), 3.65, 3.77 (each s, 15 H, 3 H, Me, and OMe), 4.40 (m, 6 H, CH_2CH_2CO and CH_2CH_2OAc), 4.89 (t, 2 H, CH_2OAc), 9.10 (s, 1 H, 4-H), 10.04, 10.10, 10.13, 10.17 (each s, 1 H, meso H).

Anal. Calcd for $C_{36}H_{40}N_4O_6$: C, 69.21; N, 6.45; N, 8.97. Found: C, 69.18; H, 6.44; N, 8.99.

2-(2-Acetoxyethyl)-4-acetyl-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (24). Copper was inserted into porphyrin **17** using copper(II) acetate in methanol/dichloromethane[49] and afforded a quantitative yield of **23**. To 319 mg of this copper complex in 50 mL of dichloromethane was added 10 mL of acetic anhydride. The mixture was cooled to 0 °C in an ice bath and then 1.0 mL of anhydrous stannic chloride was added rapidly. The mixture was stirred for 1 min before being poured over ice. Dichloromethane (50 mL) was added and the mixture was washed with 3 × 100 mL of water before the organic phase was collected and evaporated to dryness under vacuum. To the residue, dissolved in 25 mL of trifluoroacetic acid, was added 2.5 mL of concentrated sulfuric acid, and the mixture was stirred for 1 h at room temperature. The mixture was diluted with 100 mL of dichloromethane and washed with 3 × 150 mL of water. The porphyrin fraction was collected and treated with excess ethereal diazomethane. Following evaporation of the solvent, the residue was chromatographed on 1-mm thick 20 × 20 cm preparative TLC plates of silica gel G (elution with 4% methanol in dichloromethane). The porphyrin was extracted from the silica with 5% methanol in dichloromethane, and evaporation gave a residue which was crystallized from dichloromethane/heptane to give 198 mg (64% yield) of the acetylated porphyrin: mp 204–206 °C; UV-vis, λ_{max} 410 nm (ϵ 193000), 510 (10800), 550 (13150), 577 (8200), 635 (1750); ^{1}H NMR δ (ppm) 2.10 (s, 3 H, OCOMe), 3.31 (q, CH_2CO), 3.37 (s, 3 H, COMe), 3.62, 3.72, 3.97 (each s, 3 H, 12 H, 3 H, Me, and OMe), 4.37 (t, 2 H, CH_2CH_2O), 4.48 (q, 4 H, CH_2CH_2CO), 4.93 (t, 2 H, CH_2CH_2O), 10.06, 10.28, 10.87 (each s, 2 H, 1 H, 1 H, meso H).

Anal. Calcd for $C_{38}H_{42}N_4O_5$: C, 68.45; H, 6.35; N, 8.40. Found: C, 68.36; H, 6.35; N, 8.36.

4-Acetyl-2-(2-hydroxyethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (25, Undeuterated). To 91 mg of the foregoing porphyrin, **24**, in 10 mL of methanol was added 0.3 mL of concentrated sulfuric acid. After stirring at room temperature for 12 h the solution was diluted with 75 mL of dichloromethane and then washed with 3 × 100 mL of water. The organic layer was collected and evaporated under vacuum to give a residue which was shown by spectrophotometry and ^{1}H NMR analysis to be the dimethyl ketal of the acetylporphyrin **25**. Thus, the porphyrin was dissolved in 10 mL of tetrahydrofuran and stirred with 4 mL of water and 1 mL of concentrated hydrochloric acid for 20 min. The mixture was diluted with 50 mL of dichloromethane and washed with 3 × 100 mL of water before being treated with excess ethereal diazomethane and evaporated to dryness. The residue was chromatographed on neutral alumina (Brockmann Grade IV, elution with chloroform), and the red eluates were evaporated to give a residue which was recrystallized from dichloromethane/heptane to give 80 mg (94% yield) of the required 2-(2-hydroxyethyl)porphyrin: mp 276–278 °C (lit.[40c] mp 277–279 °C); ^{1}H NMR δ (ppm) 3.24 (t, 6 H, CH_2OH and CH_2CO), 3.34 (s, 3 H, COMe), 3.60, 3.64, 3.92, 4.42 (6 H, 6 H, 3 H, 3 H, Me, and OMe), 4.35 (m, 6 H, CH_2CH_2CO and CH_2CH_2O), 9.92, 10.06, 10.69, 11.19 (each s, 1 H, meso H).

Anal. Calcd for $C_{36}H_{40}N_4O_6$: C, 69.21; H, 6.45; N, 8.97. Found: C, 69.34; H, 6.48; N, 9.01.

When the reaction was repeated using D_2SO_4/MeOD, the product, **25** was identical with that described above, except that the ^{1}H NMR resonance at δ 3.34 was absent (see below).

2-(2-Chloroethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-4-vinylporphyrin (21, Undeuterated). To 68 mg of the foregoing porphyrin in 40 mL of dichloromethane was added 5 mL of ice-cold methanol containing 75 mg of sodium borohydride. The mixture was stirred for 1 h at room temperature, after which time TLC monitoring indicated reduction of the acetyl group to be complete. The mixture was washed with 30 mL of 0.1 M hydrochloric acid, followed by two washings with 100 mL of water. The organic phase was collected and evaporated to dryness under vacuum; then the residue was dissolved in 10 mL of dry

(49) Reference 27, p 798.

dimethylformamide, to which was added 1 mL of benzoyl chloride. The mixture was heated at 98 °C for 1.5 h under nitrogen, allowed to cool, and then diluted with 75 mL of dichloromethane and washed with 50 mL of 1 M sodium hydroxide, and then 2 × 75 mL of water. The organic phase was evaporated to dryness and the residue was chromatographed on 1-mm thick 20 × 20 cm preparative silica gel G plates (elution with 2% methanol in dichloromethane). The porphyrin was recovered from the silica by washing with 3% methanol in dichloromethane, which was evaporated, and the residue was recrystallized from dichloromethane/heptane to give 51 mg (74% yield) of the vinylporphyrin: mp 250–252 °C (lit.[40c] mp 250–252 °C); ^{1}H NMR δ (ppm) 3.33 (t, 4 H, CH_2CO), 3.69, 3.80 (each s, 15 H, 3 H, Me, and OMe), 4.50 (m, 8 H, CH_2CH_2CO and CH_2CH_2Cl), 6.24 (d, 1 H, cis β-vinyl CH, J = 12 Hz), 6.42 (d, 1 H, trans β-vinyl CH, J = 18 Hz), 8.31 (m, 1 H, α-vinyl CH), 10.09, 10.14, 10.27 (each s, 1 H, 2 H, 1 H, meso H).

Anal. Calcd for $C_{36}H_{39}ClN_4O_4$: C, 68.94; H, 6.27; N, 8.93. Found: C, 68.57; H, 6.22; N, 8.81.

2-(2-Chloroethyl)-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-4-(α,β,α-trideuteriovinyl)porphyrin (21). To 91 mg of porphyrin **24** in 10 mL of MeOD was added 9 drops of concentrated D_2SO_4. The mixture was stirred overnight at room temperature in a well-stoppered flask. After dilution with 50 mL of dichloromethane, the mixture was washed with 2 × 50 mL of water, and, as in the unlabeled case, TLC and spectrophotometry indicated the presence of considerable quantities of the dimethyl ketal. The organic phase was therefore evaporated to dryness and the residue was dissolved in 50 mL of tetrahydrofuran containing 4 mL of D_2O and 1 mL of 20% DCl in D_2O. The mixture was stirred for 20 min at room temperature before being diluted with 100 mL of dichloromethane and washed with 2 × 100 mL of water. The organic phase was treated briefly with excess ethereal diazomethane, then evaporated to dryness to give a residue which was dissolved in 40 mL of dichloromethane and treated with 5 mL of ice-cold MeOD containing 75 mg of sodium borodeuteride. Reduction of the acetyl was complete after stirring for 1 h at room temperature, so the solution was diluted with 50 mL of dichloromethane which was washed with 50 mL of 0.1 M hydrochloric acid, followed by 2 × 75 mL of water. The organic fraction was collected, the solvent evaporated under reduced pressure, and the residue dissolved in 10 mL of dry dimethylformamide, to which was then added 1 mL of benzoyl chloride. After heating at 98 °C for 1.5 h under nitrogen, the solution was cooled, diluted with 75 mL of dichloromethane, and then washed successively with 75 mL of 2 M sodium hydroxide and 2 × 100 mL of water. The solvent was removed under vacuum and the residue was chromatographed on 1-mm thick 20 × 20 cm preparative silica gel G TLC plates (elution with 2% methanol in dichloromethane); the principal band was washed from the silica with 3% methanol in dichloromethane, then evaporated to give a red residue which was recrystallized from dichloromethane/methanol to give 66 mg (77% yield) of the deuterated porphyrin, mp 248–250 °C (lit.[40c] mp 250–252 °C, undeuterated). The ^{1}H NMR spectrum was identical with that for the unlabeled material described above, except that the resonance at δ 8.31 was absent, and those at δ 6.24 and 6.42 were reduced to 30% intensity, indicating 70% deuteration at the β-vinyl methylene, and 100% deuteration at the α-vinyl methine.

6,7-Bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-vinyl-4-(α,β,-β-trideuteriovinyl)porphyrin (27). The foregoing porphyrin **21** (62 mg) was dissolved in 25 mL of pyridine which had previously been refluxed under nitrogen for 30 min; this mixture was then heated under nitrogen at 105 °C and first treated with 4.0 mL of water, followed after 5 min by another 4.5 mL of 3% sodium hydroxide solution. The mixture was stirred at 105 °C for 2.5 h before being cooled to room temperature and diluted with 50 mL of dichloromethane and 50 mL of tetrahydrofuran. The organic phase was washed with 2 × 100 mL of 2 M hydrochloric acid and finally with 100 mL of water. After brief treatment with excess ethereal diazomethane, the solution was evaporated to dryness under reduced pressure and the residue was chromatographed in the dark on 1-mm thick 20 × 20 cm preparative silica gel G TLC plates (elution with 2% methanol in dichloromethane). The major band was removed from the silica with 3% methanol in dichloromethane, which was evaporated to give a red residue, crystallized from dichloromethane/heptane to give 49 mg (83% yield) of the 4-vinyl-labeled protoporphyrin IX dimethyl ester, mp 225–227 °C (lit.[50] mp 228–229 °C, undeuterated). Apart from peak absences in the ^{1}H NMR spectrum, this material was identical with an authentic sample of protoporphyrin IX dimethyl ester (**1**): ^{1}H NMR δ (ppm) 3.27 (t, 4 H, CH_2CO), 3.60, 3.63, 3.71 (each s, 3 H, 3 H, 6 H, Me), 3.65 (s, 6 H, OMe), 4.40 (t, 4 H, CH_2CH_2CO), 6.19 (*d*, 1.32 H, cis β-vinyl CH, J = 12 Hz, 68% D), 6.37 (d, 1.29 H, trans β-vinyl CH, J = 18 Hz, 71% D), 8.29 (m, 1 H, α-vinyl CH, 100% D at position 4), 10.04, 10.09, 10.17, 10.23 (each s, 1 H, meso H).

4-(2-Chloroethyl)-2-formyl-6,7-bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphyrin (35). To 2.0 mL (11.27 mM, 1.77 g) of *N,N*-diisobutylformamide was added rapidly, but dropwise, 100 mL (10.98 mM, 1.68 g) of phosphoryl chloride; the mixture was stirred at room temperature for 40 min. To the resulting viscous imine salt were added 115 mg of copper(II) 4-(2-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**32**) (obtained in quantitative yield from the free base porphyrin **31** using the copper acetate metal insertion procedure[49]) and 50 mL of 1,2-dichloroethane. The mixture was stirred under nitrogen at 70 °C for 7 h, after which time TLC analysis indicated that the reaction was not proceeding any further. The mixture was therefore cooled in an ice bath and a saturated solution of sodium bicarbonate was added cautiously, after which the mixture was allowed to warm to room temperature, with stirring, overnight. The organic phase was separated, diluted with 50 mL of dichloromethane, and then washed with 2 × 100 mL of water. The organic layer was evaporated under reduced pressure and the residue was chromatographed on 1-mm thick 20 × 20 cm preparative silica gel G plates (elution with 2.5% methanol in dichloromethane), to give three major bands. The most mobile fraction appeared bright red; the least mobile fraction, the most abundant, trailed slightly into the middle band and was green in color. The middle band, clearly separated from the upper fraction, was bright green in color. The bands were recovered from the silica by washing with 5% methanol in dichloromethane, and, following evaporation of the solvent, the residues were demetalated by stirring in 15 mL of trifluoroacetic acid containing 1.5 mL of concentrated sulfuric acid. After 1 h at room temperature, the solutions were diluted with 100 mL of dichloromethane and washed with 3 × 100 mL of water; the separated organic phases were treated with excess ethereal diazomethane. The solvents were removed immediately and the three residues were then chromatographed once more on 1-mm thick 20 × 20 cm preparative silica gel G TLC plates (elution with 2.5% methanol in dichloromethane). After the usual workup, the residues were crystallized from dichloromethane/heptane to give, as the most mobile fraction, 17 mg (16% yield) of 4-(2-chloroethyl)deuteroporphyrin IX dimethyl ester (**33**). From the middle fraction was isolated 23 mg (20% yield) of what is proposed to be α-*meso*-formyl-4-(2-chloroethyl)deuteroporphyrin IX dimethyl ester (**36**). Finally, the least mobile fraction yielded 49 mg (44% yield) of 4-(2-chloroethyl)-2-formyldeuteroporphyrin IX dimethyl ester (**35**).

Least Mobile Fraction: 4-(2-Chloroethyl)-2-formyldeuteroporphyrin IX Dimethyl Ester (35): mp 229–231 °C (lit.[40a] mp 228–230 °C); ^{1}H NMR δ, (ppm), 3.27 (m, 4 H, CH_2CO), 3.54, 3.63, 3.70, 3.90 (each s, 3 H, Me), 3.67 (s, 6 H, OMe), 4.31 (t, 4 H, CH_2CH_2Cl), 4.44, 4.53 (each t, 4 H, CH_2CH_2CO), 9.88, 10.00, 10.08, 10.95 (each s, 1 H, meso H), 11.42 (s, 1 H, CHO).

Middle Fraction: α-*meso*-Formyl-4-(2-chloroethyl)deuteroporphyrin IX Dimethyl Ester (36): mp 189–191 °C; λ_{max} 411 nm (ε 145 100), 510 (8800), 558 (8300), 646 (7500); ^{1}H NMR δ (ppm) 3.23 (m, 4 H, CH_2CO), 3.50, 3.56, 3.59, 3.63 (each s, 3 H, Me), 3.67, 3.69 (each s, 3 H, OMe), 4.25–4.46 (m, 8 H, CH_2CH_2CO and CH_2CH_2Cl), 9.70 (s, 1 H, 2-H), 9.97, 10.00, 10.12 (each s, 1 H, meso H), 12.54 (s, 1 H, CHO).

Anal. Calcd for $C_{35}H_{37}ClN_4O_5$: C, 66.82; H, 6.09; N, 8.90. Found: C, 66.93; H, 6.09; N, 8.69.

2-Formyl-4-vinyldeuteroporphyrin IX Dimethyl Ester, "*Spirographis* Porphyrin Dimethyl Ester" (28). The foregoing 2-formylporphyrin, **35** (72.1 mg), in dry benzene (50 mL) under nitrogen was brought to reflux and then treated with ethylene glycol (1.25 mL) and *p*-toluenesulfonic acid (9.0 mg). Progress of the reaction was followed by spectrophotometry, using the change in absorption spectrum from "rhodo" to "etio" type; the reaction was complete after 45 min. The mixture was diluted with dichloromethane (100 mL), washed with water (3 × 250 mL), dried (Na_2SO_4), and evaporated to dryness to give a residue which was dried further under vacuum. The resulting acetal, **37** [^{1}H NMR δ (ppm) −3.76 (br s, 2 H, NH), 3.28, 3.29 (each t, 2 H, CH_2CO), 3.61, 3.64, 3.66, 3.67, 3.69, 3.77 (each s, 3 H, Me and OMe), 4.31, 4.38, 4.42, 4.53, 4.55, 4.80 (each t, 2 H, CH_2s in acetal ring, propionates and chloroethyl), 7.39 (s, 1 H, acetal CH), 9.95, 10.07, 10.13, 10.43 (each s, 1 H, meso H)], was taken up in pyridine (30 mL) which had previously been refluxed under nitrogen for 30 min, to which were added water (5.4 mL) and 3% aqueous sodium hydroxide solution (6.4 mL). The solution was refluxed under nitrogen for 2.5 h, before addition of 25% acetic acid in water (6.4 mL), evaporation to a minimum volume, and chasing with toluene to remove most of the pyridine. Addition of water caused precipitation of the crude product (**38**) which was filtered off, washed with water, and then dissolved in 5% sulfuric acid in methanol (20 mL). After standing overnight, the reaction mixture was worked up by dilution with dichloromethane (200 mL), washing with water (3 × 250 mL), drying

(50) Fischer, H.; Orth, H. "Die Chemie des Pyrrols"; Akademische Verlag: Leipzig, 1937; Vol. II, Part 1, p 401.

(Na_2SO_4), and evaporation to dryness. The residue was crystallized from dichloromethane–methanol to give 51.4 mg (75%) of the required porphyrin, mp 278.5–279.5 °C (lit.[41a] mp 278 °C). This material was shown, by TLC and mixture melting point analysis, to be identical with an authentic sample of *Spirographis* porphyrin dimethyl ester: λ_{max} 421 nm (ε 138 000), 516 (11 400), 556 (15 100), 582 (9400), 642 (2500); ^1H NMR spectrum δ (ppm) −4.35 (br s, 2 H, NH), 3.22 (t, 4 H, CH_2CH_2CO), 3.48, 3.55, 3.64, 3.67, 3.72, 3.73 (each s, 3 H, Me and OMe), 4.27, 4.34 (each t, 2 H, CH_2CH_2CO), 6.26, 6.40 (each d, 1 H, α-vinyl CH), 8.20 (dd, 2 H, β-vinyl CH_2), 9.65, 9.76, 9.80, 10.55 (each s, 1 H, meso H), 11.23 (s, 1 H, CHO).

Anal. Calcd for $C_{35}H_{36}N_4O_5$: C, 70.3; H, 6.12; N, 9.45. Found: C, 71.06; H, 6.45; N, 9.08.

2,4-Diformyldeuteroporphyrin IX Dimethyl Ester (40), 2-Formyl-4-vinyldeuteroporphyrin IX Dimethyl Ester ("*Spirographis* Porphyrin Dimethyl Ester" (28)), and 4-Formyl-2-vinyldeuteroporphyrin IX Dimethyl Ester ("Iso-*Spirographis* Porphyrin Dimethyl Ester" (39)) by Ozonolysis of Protoporphyrin IX Dimethyl Ester[44] (1). Protoporphyrin IX dimethyl ester (4 g) in 4.6 L of chloroform and 390 mL of methanol was treated with 8 mL of concentrated sulfuric acid. The resulting deep red solution was cooled to −63 °C (chloroform/dry ice bath), the flask was covered with aluminum foil, and 1 equiv of ozone was passed through the stirred solution. The mixture was stirred for an addition 15 min and then degassed with a stream of nitrogen gas at −63 °C for 45 min. To the stirred mixture were then added 7 g of sodium bicarbonate and 14 mL of dimethyl sulfide, and the mixture was stirred, each time for 1 h, at −63, 0, and finally 20 °C. The chloroform was evaporated under vacuum and the resulting slurry was poured into 1 L of water and extracted with 5 × 100 mL of chloroform. The combined organic phases were washed with 2 × 10 mL of water, dried (Na_2SO_4), and then evaporated to dryness. The residue was separated by chromatography on neutral alumina (Brockmann Grade IV, elution initially with dichloromethane, and then with 5% methanol in dichloromethane), and the eluates were evaporated to give, from the least mobile band, 2,4-diformyldeuteroporphyrin IX dimethyl ester, 940 mg (23.4%), recrystallized from dichloromethane/hexane, mp 284–286 °C (lit.[51] mp 284–286 °C). The more mobile band was shown by analytical HPLC (microporasil column, elution with 1.2% tetrahydrofuran in dichloromethane) to consist of two closely running monoformylmonovinyl isomers, so the residue was subjected to MPLC at 100 psi (see earlier for description of system) on Whatman LPS-1 silica. The elution solvent was dichloromethane/tetrahydrofuran/isopropyl alcohol (98.4:1.5:0.1), and eluted porphyrins were monitored at 405 nm. The pure isomeric fractions were evaporated to dryness and afforded, as the most mobile fraction, 2-formyl-4-vinyldeuteroporphyrin IX dimethyl ester (*Spirographis* porphyrin dimethyl ester, **28**) (80 mg, 2%), crystallized from dichloromethane/hexane, with mp 279 °C (lit.[41a] mp 278 °C). The least mobile fraction gave 4-formyl-2-vinyldeuteroporphyrin IX dimethyl ester (iso-*Spirographis* porphyrin dimethyl ester, **39**), (140 mg, 3.5%), crystallized from dichloromethane/hexane, with mp 225–227 °C, (lit.[41a] mp 225–227 °C). Analytical HPLC (see above) showed the two isomers to be uncontaminated with each other, and the identities of the individual compounds were established by melting point and mixture melting point comparisons, and finally by 360-Mz ^1H NMR spectroscopy.

6,7-Bis(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2,4-bis([$^{13}C_2$]-vinyl)porphyrin (43). Zinc was inserted in quantitative yield into 2,4-diformyldeuteroporphyrin IX dimethyl ester (**40**) using the zinc(II) acetate method.[49] Dry [^{13}C]methyltriphenylphosphonium iodide (mp 182–184 °C, lit.[52] mp 187–189 °C, unlabeled) (67 mg) (prepared[53] from commercially available 99%-enriched $^{13}CH_3I$ and triphenylphosphine) in dry tetrahydrofuran (25 mL) and diisopropylamine (0.55 mL) were treated with *n*-butyllithium in *n*-hexane (0.14 mL, 2.3 M). The resulting ylide was stirred under nitrogen gas and zinc(II) 2,4-diformyldeuteroporphyrin IX dimethyl ester (54 mg) was added. After 5 min the solution was evaporated to dryness to give a residue which was taken into dichloromethane (50 mL), filtered through anhydrous sodium sulfate, and then evaporated again to dryness. The residue was dissolved in trifluoroacetic acid (5 mL) and after 5 min was diluted with dichloromethane (50 mL) and washed twice with water (50 mL). The organic phase was evaporated to dryness and the residue was chromatographed on alumina (Brockmann Grade III, elution with 20% toluene in dichloromethane). The red eluates were evaporated to dryness to furnish a residue which was recrystallized from dichloromethane/methanol to give 26.9 mg (51%) of the doubly labeled protoporphyrin IX dimethyl ester, mp 208–210 °C (lit.[54] mp 228–229 °C, unlabeled). The proton NMR spectrum was identical with that of authentic protoporphyrin IX dimethyl ester, except in the vinyl region, where the $=CH_2$ resonances appeared at 6.18 (dd, J = 11.4 and 159.12 Hz) and 6.37 ppm (dd, J = 17.8 and 156.5 Hz). The carbon-13 NMR spectrum showed a single enhanced resonance at 120.91 ppm, which split into a triplet (J = 158.8 Hz) in the proton-coupled mode.

6,7-Di(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-([$^{13}C_2$]vinyl)-4-vinylporphyrin Dimethyl Ester (41). This porphyrin was likewise prepared from the zinc(II) complex of 2-formyl-4-vinyldeuteroporphyrin IX dimethyl ester (**28**) (93 mg) and [^{13}C]methyltriphenylphosphonium iodide (93 mg). The product **41** (59.7 mg; 52%) was crystallized from dichloromethane/methanol and had mp 203–205 °C (lit.[54] mp 228–229 °C, unlabeled). The proton NMR spectrum was identical with that of natural material, except that in the vinyl proton region, one of the vinyl sets of resonances was observed as a carbon-coupled multiplet at 6.28 (dd, J = 11.50 and 158.9 Hz) and 6.39 ppm (dd, J = 18.13 and 156.5 Hz). In the proton-decoupled carbon-13 NMR spectrum a singlet enhanced signal was observed at 120.92 ppm, and in the proton coupled mode this was split into a triplet, J = 157.90 Hz.

6,7-Di(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-vinyl-4-([$^{13}C_2$]-vinyl)porphyrin Dimethyl Ester (42). In a similar manner, this carbon-13 monolabeled porphyrin was prepared from the zinc(II) complex of 4-formyl-2-vinyldeuteroporphyrin IX dimethyl ester (**39**) (108 mg) and 99% enriched [^{13}C]methyltriphenylphosphonium iodide (167 mg). The product (39 mg, 39%) was recrystallized from dichloromethane/methanol and had mp 205–207 °C (lit.[54] mp 228–229 °C). The proton NMR spectrum was identical with that of authentic protoporphyrin IX dimethyl ester, except that one set of vinyl resonances experienced a carbon-13 coupling at 6.28 (dd, J = 11.63 and 159.00 Hz) and 6.36 ppm (dd, J = 17.8 and 156.30 Hz). The carbon-13 NMR spectrum showed a single enhanced resonance at 120.89 ppm which appeared as a triplet (J = 158.45 Hz) in the proton-coupled mode.

2,4-Di([1-^{13}C]acetyl)deuteroporphyrin IX Dimethyl Ester (47). Anhydrous (99% enriched) sodium [1-^{13}C] acetate (2.0 g) was dried at 80 °C (0.5 mmHg) and then suspended in 100 mL of dry dichloromethane; after cooling to 0 °C in an ice bath, 3 mL of oxalyl chloride was added. The suspension was stirred at 0 °C for 3 h, during which time the solid appeared to change in texture. Deuterohemin dimethyl ester (100 mg) was then added, followed by dropwise addition of 2 mL of stannic chloride. The mixture was kept in the dark, under nitrogen at 0 °C for 12 h, and then poured into 100 mL of ice cold water which was extracted with 3 × 100 mL of chloroform. The combined organic phases were dried (Na_2SO_4) and evaporated to dryness to give a residue which was dissolved in 100 mL of dry methanol containing 1.2 g of powdered ferrous sulfate. Hydrogen chloride gas was bubbled through the solution for 25 min, after which time spectrophotometry showed a pure, two-banded, porphyrin dication spectrum. The solution was therefore cooled in an ice bath and poured into 300 mL of ice-cold water. After extraction with 3 × 100 mL of chloroform, the extracts were washed successively with water, saturated aqueous sodium bicarbonate, and finally water again before being dried (Na_2SO_4) and evaporated to give a residue. This was chromatographed on neutral alumina (Brockmann Grade III, elution with dichloromethane) and gave three major bands. The least polar band afforded deuteroporphyrin IX dimethyl ester (**7**) (22 mg, 20%), crystallized from dichloromethane/hexane, with mp 223–224 °C (lit.[28] mp 224 °C). The middle band was further purified on 1-mm thick 20 × 20 cm preparative TLC plates of silica gel (elution with 2% tetrahydrofuran in dichloromethane) and afforded two isomeric [^{13}C]monoacetyldeuteroporphyrins **45** and **46**; the less polar of the isomers, after recrystallization from dichloromethane/hexane, mp 236.5–237.5 °C (17 mg, 16%), was identified as the carbon-13 labeled 2-acetyldeuteroporphyrin IX dimethyl ester (**45**) (lit.[31] mp 238.5–240.5 °C, unlabeled). The more polar isomer, mp 209.5–210.5 °C, recrystallized also from dichloromethane/hexane (21 mg, 20%), was similarly identified as carbon-13 labeled 4-acetyldeuteroporphyrin IX dimethyl ester (**46**) (lit.[31] mp 211–213 °C, unlabeled). The least polar fraction from the alumina chromatography, obtained after changing the elution solvent to chloroform, was identified as doubly labeled 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**47**), mp 239–240 °C (lit.[28] mp 244 °C), and was obtained in 21% yield (22 mg), after recrystallization from dichloromethane/hexane. The carbon-13 NMR spectrum at 50 MHz showed one enriched carbon resonance at 200.00 ppm: ^1H NMR δ (ppm) −3.56 (br s, 2 H, NH), 3.25 (t, 4 H, CH_2CO), 3.7–3.8 (m, 6 H, COMe), 3.58, 3.60, 3.85, 3.90 (each s, 3 H, Me), 3.70 (s, 6 H, OMe), 4.40 (t, 4 H, CH_2CH_2CO), 9.80, 9.91, 10.62, 10.80 (each s, 1 H, meso H).

(51) Caughey, W. S.; Alben, J. O.; Fujimoto, W. Y.; York, J. L. *J. Org. Chem.* **1966**, *31*, 2631–2640.

(52) Hoffmann, H.; Grunewald, R.; Horner, L. *Chem. Ber.* **1960**, *93*, 861–865.

(53) Monson, R. S. "Advanced Organic Synthesis: Methods and Technique"; Academic Press: New York, 1971; p 105.

(54) Reference 27, p 772.

2,4-Di([1-^{13}C]-1-hydroxyethyl)deuteroporphyrin IX Dimethyl Ester (48). The foregoing carbon-13 enriched diacetylporphyrin (20 mg) in 50 mL of chloroform was treated with a solution of 20 mg of sodium borohydride in 2 mL of ice-cold methanol. The mixture was stirred at room temperature for 25 min, after which time it was determined by analytical TLC that reaction was complete. Spectrophotometry also showed a hypsochromic shift in the 639-nm band to 619 nm. Excess hydride was decomposed by addition of 150 mL of 0.04 M hydrochloric acid and the solution was then carefully neutralized with aqueuous ammonia. The organic layer was separated and evaporated to dryness to give a residue which was recrystallized from dichloromethane/hexane to give 18 mg (90%) of doubly labeled hematoporphyrin IX dimethyl ester (**48**), mp 219-220 °C (lit.[50] mp 223 °C). The carbon-13 NMR spectrum at 50 MHz showed a single enriched peak at 62.50 ppm which became a doublet in the proton-coupled mode, with J_{C-H} = 140.6 Hz.

2,4-Di([1-^{13}C]vinyl)deuteroporphyrin IX Dimethyl Ester (44). The carbon-13 enriched hematoporphyrin (**48**) (18 mg) was dissolved in 6 mL of dimethylformamide containing 1 mL of benzoyl chloride and heated at 90 °C for 1.5 h. After this time 2 mL of triethylamine was added to the warm solution, followed by 15 mL of water and 7 mL of methanol. The mixture was shaken to precipitate the product, which was collected by filtration through Celite, and then washed with 10 mL of water and redissolved by addition of 20 mL of chloroform and then collection by suction through the Celite. The organic phase was evaporated and the residue was purified by chromatography on 1-mm thick 20 × 20 cm preparative silica gel TLC plates (elution with 2% methanol in dichloromethane). The porphyrin was recovered from the silica by washing with 3% methanol in dichloromethane, and, after crystallization from dichloromethane/hexane, a quantitative yield of doubly labeled protoporphyrin IX dimethyl ester (**44**) was obtained, with mp 215-217 °C (lit.[50] mp 228-229 °C, unlabeled). The carbon-13 ^{1}H NMR spectrum showed a single enhanced peak at 130.32 ppm, and in the proton-coupled mode the J_{C-H} was 153.4 Hz: ^{1}H NMR δ (ppm) -3.70 (br s, 2 H, NH), 3.30 (m, 4 H, CH_2CO), 3.68, 3.69, 3.76, 3.77, 3.93 (each s, 3 H, 3 H, 3 H, 3 H, 6 H, Me, and OMe), 4.23, 4.43 (each m, 2 H, CH_2CH_2CO), 6.21, 6.41 each d, 2 H, $CH{=}CH_2$), 8.10, 8.52 (each m, 1 H, $CH{=}CH_2$), 10.05, 10.15, 10.21, 10.30 (each s, 1 H, meso H).

Typical Iron Insertion Procedure: Synthesis of Hemin Chloride Dimethyl Ester. To a three-necked, 100-mL round-bottom flask, equipped with a reflux condenser (topped with a nitrogen gas inlet), a pressure-equilibrated dropping funnel (topped with a septum cap), and a nitrogen gas escape tube (with a glycerol bubbler), was added 25 mL of acetonitrile. After refluxing the acetonitrile for 30 min with vigorous stirring (bar magnet) and under a stream of nitrogen, 150 mg of ferrous chloride hydrate was added, and this dissolved with the heat. The mixture was cooled to 50 °C and a solution of 110 mg of protoporphyrin IX dimethyl ester (**1**) in nitrogen-purged chloroform (12 mL) was transferred through the septum cap into the dropping funnel. Though some of the ferrous salt had precipitated, the porphyrin solution was added to the stirred ferrous chloride solution at a rate of 2 mL per min. After complete addition, the mixture was stirred for a further 10 min under nitrogen before being exposed to air. The resulting brown solution was then diluted with 125 mL of dichloromethane and washed with 125 mL of 0.2 M hydrochloric acid, and then with 100 mL of water. The organic phase was collected, the solvent evaporated, and the residue sampled by analytical silica gel TLC. The chromatogram indicated that no free base **1** was present and that no ester hydrolysis had occurred. The residue was chromatographed (optional) on neutral alumina (Brockmann Grade III, elution with chloroform containing 0.5% methanol). The major fraction was collected, washed with 0.1 M hydrochloric acid, and then evaporated to dryness to give a brown residue which was crystallized from dichloromethane/heptane to give hemin chloride dimethyl ester (116 mg, 92%), identical by TLC and ^{1}H NMR spectroscopy (as the cyanoferrihemin) with an authentic sample.

Typical Ester Hydrolysis: Synthesis of Hemin (5). The foregoing hemin dimethyl ester (50 mg) was dissolved in 15 mL of a solution made by mixing 95 mL of methanol, 5 mL of water, and 1 g of potassium hydroxide. The solution was refluxed for 5 h at 60 °C under an atmosphere of dry nitrogen. The warm solution was then diluted with 20 mL of methylene chloride and washed with 2 × 50 mL of 0.2 M hydrochloric acid. The organic phase was collected, dried (Na_2SO_4), and evaporated to dryness to give a residue which was recrystallized from tetrahydrofuran to give 40 mg (80% yield) of hemin, identical with an authentic sample by TLC, spectrophotometry, and ^{1}H NMR spectroscopy (in D_2O, as the dicyanohemin).

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Registry No. 1, 5522-66-7; **2**, 10591-31-8; **3**, 13003-76-4; **4**, 15295-25-7; **5**, 16009-13-5; **8**, 14376-24-0; **10**, 74822-24-5; **11**, 74822-25-6; **12**, 63148-21-0; **13**, 18210-87-2; **14**, 87191-13-7; **15**, 87191-14-8; **16**, 52459-76-4; **17**, 52459-74-2; **21**, 87206-75-5; **23**, 87191-08-0; **23** acetyl derivative, 87206-74-4; **24**, 87191-15-9; **24** dimethyl ketal derivative, 87191-19-3; **25**, 87191-17-1; **25** undeuterated, 58684-37-0; **25** dimethyl ketal derivative, 87191-16-0; **26**, 87191-20-6; **26** undeuterated, 87191-18-2; **27**, 87206-76-6; **27** undeuterated, 58684-43-8; **28**, 10200-04-1; **28** zinc complex, 61577-42-2; **29**, 15295-26-8; **30**, 17467-73-1; **31**, 52459-75-3; **32**, 87191-09-1; **33**, 52459-77-5; **33** copper complex, 87191-11-5; **34**, 87191-12-6; **35**, 52459-79-7; **36**, 87206-77-7; **37**, 87191-21-7; **38**, 87191-22-8; w39, 13187-15-0; **40**, 15341-25-0; **40** zinc complex, 87191-10-4; **41**, 5522-66-7; **42**, 87191-23-9; **43**, 87226-18-4; **44**, 87191-26-2; **45**, 87191-24-0; **46**, 87206-79-9; **47**, 87206-78-8; **48**, 87191-25-1; deuterohemin dimethyl ether, 19442-32-1; sodium [1-^{13}C]acetate, 23424-28-4; hemin chloride dimethyl ester, 15741-03-4.

Ab Initio Investigation on the Lowest Singlet and Triplet State of Si_2H_2

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Abstract: Ab initio SCF and electron correlation calculations are reported for the Si_2H_2 system. In the case of the singlet ground state the global minimum is a nonplanar bridged structure (IV) followed by H_2SiSi (II) and trans-bent HSiSiH (I). Both H_2SiSi and trans-bent HSiSiH are predicted to be local minima. Stability differences ($\Delta H°_{298.16}$) obtained from our most extensive calculations are II/IV 11.8 kcal/mol and I/IV 14.3 kal/mol. In the triplet case H_2SiSi (II) is the global minimum. Trans bent HSiSiH (I) and a planar bridged structure (III) are local minima. The following stability differences were obtained: I/II 1.7 kcal/mol, III/II 19.7 kcal/mol.

1. Introduction

Compounds containing silicon involved in multiple bonds have always attracted the interest of theoretical and experimental chemists. However, despite the numerous experimental investigations, direct structural evidence thereof is rather scarce. In recent years theoretical methods have been developed to a degree which allows in many cases (especially for closed-shell ground states) the accurate prediction of structures and stability differences. In the case of silaolefins the interrelation between theory

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