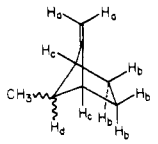
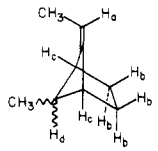
Table III

compd	NMR, δ
	
2 (exo)	4.43 (s, H_a), 1.64 (s~d, H_b), 2.42 (~d, H_c), 1.34[a] (~q, H_d), 1.32[a] (d, CH_3)
3 (endo)	4.24 (s, H_a), 1.53 (s~d, H_b), 0.73 (d, CH_3), 2.55 (q, H_c), 1.09 (q, H_d)
	
5-methyl-6-ethylidene-bicyclo[2.1.1]hexene[18]	
exo	4.85 (q, H_a), 2.0–1.6 (4 H_b + H_d), 2.60–2.35 (H_c), 1.52 and 1.05 (2 d, 2 CH_3)
endo	4.65 (q, H_a), 2.3–1.5 (4 H_b + H_d), 2.75–2.5 (H_c), 1.50 and 0.72 (2d, 2 CH_3)

[a] Overlap.

~70%. Compound 6 was purified by preparative GLC on the column B at 40 °C. The NMR spectrum showed absorption at δ 5.8–4.6 (m, 6 H), 2.1 (m, 3 H), and 1.0 (d, CH_3) and the terminal vinyl absorptions were extremely similar to those of 4-methyl-1-pentene and 4-methyl-1-hexene.

Photolyses. The benzene-sensitized photolyses of 1 were carried out in a quartz cell (79.5 cm) with Vycor windows. The low-pressure mercury lamp (a helical Hanovia 69A-1, 7.56 × 13.86 cm) was positioned so as to pass light to the cell through a Vycor window at maximum efficiency. With this system, it was not necessary to remove the cell from the vacuum line during photolyses. Nitrogen gas was passed over the cell to remove ozone as formed and for cooling. In a typical photolysis, 5 μL of 1 and 5.5 μL of benzene (99.9% pure) were mixed. After irradiation the mixture was analyzed by GLC on the following aluminum columns ($^1/_8$ in. o.d.): D, 15% TCEP on Chromosorb P (80–100 mesh); E, 15% TEG on Chromosorb P (80–100 mesh); F, DURAPAK (*n*-octane) on Porasil C (120–150 mesh); G, 10% Carbowax 20M on Chromosorb W (100–120 mesh).

Identification of 2 and 3 was largely based on their mass and NMR spectra. The NMR spectra are also in good agreement with those of model compounds such as 5-methylenebicyclo[2.1.1]-hexane[18] and *exo*- and *endo*-5-methyl-6-ethylidenebicyclo-[2.1.1]hexane[18] (Table III). Compounds 4 and 6 were identified on the basis of identity of GLC retention times (on three columns) (Table I) with those of independently synthesized samples. Identification of 5 was based on the identity of GLC retention times with those of an authentic sample isolated from the pyrolysate of 1. Compound 7 was identified on the basis of the identity of GLC retention times (on the four columns, Table I) with those of the Cope rearrangement product which was obtained by the short-contact pyrolysis of 6.

Pyrolysis. The pyrolyses of 1-hexen-5-yn-3-ol were carried out in a cylindrical Pyrex tube (3.0 × 10.2 cm) in which nitrogen gas was passed. Pyrex brand glass wool or helices (i.d. $^1/_8$ in.) was used as a packing agent. Temperatures were measured by a copper–constantan thermocouple. All analyses of pyrolysate were made by GLC using column D at 90 °C. Pyrolysates were analyzed by GLC, using column C at 60 °C and column D at 40 °C.

(18) W. R. Roth and K. Enderer, *Justus Liebigs Ann. Chem.*, **733**, 44 (1970).

Actinometry. Benzene–*cis*-2-butene actinometry was used to determine quantum yields of products in the benzene-sensitized photolysis of *cis*-1,2,6-octatriene at 2537 Å. Photolyses of *cis*-2-butene with benzene were carried out at butene and benzene pressure at 8.45[6] and 17 mmHg,[5] respectively. The triene was photolyzed under identical conditions with the same amount of benzene. All analyses were performed by GLC on a 15% silver nitrate/TEG-25% Ucon 50 column at ambient temperature.

Acknowledgment. I acknowledge Dr. Harold R. Ward for his useful advice and Mr. Benjamin Karan for his drawings. This research was supported in part by NIH MBS Grant 1-S06RR08171-02.

Registry No. 1, 76963-27-4; 2, 76986-45-3; 3, 77027-46-4; 4, 76963-28-5; 5, 76963-29-6; 6, 76963-30-9; 7, 22701-13-9; 8, 76963-31-0; 11, 76963-32-1; 4,5-hexadienal, 20521-51-1; 1-hexen-5-yn-3-ol, 1573-66-6; *cis*-2-hexene, 7688-21-3; *trans*-2-hexene, 4050-45-7.

Syntheses of Protoporphyrin IX Analogues Bearing Acetic and Butyric Side Chains

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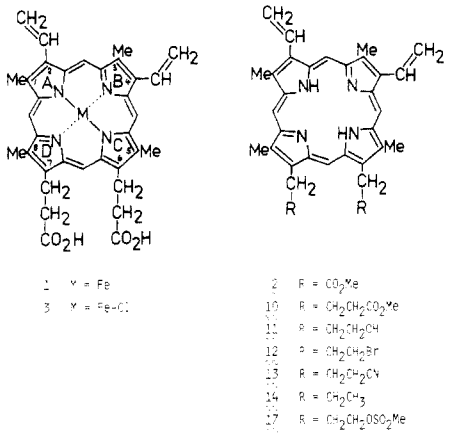
Protoheme (1) is the prosthetic group in myoglobins, hemoglobins, and several other types of heme protein.[1] X-ray studies have shown[2] that the vinyl-bearing rings are the most deeply embedded in the protein pockets of myoglobin and hemoglobin and that the 6 and 7 propionic acid groups on rings C and D are consequently pointing to the outside of the protein cleft. It seems likely that the length of the 6 and 7 substituent side chains might affect physiological action because if the carboxylates are to be situated at the polar edge of the heme pocket then the number of connecting carbons will affect the depth of the heme within the cleft, and therefore the proximity of the iron-binding histidine imidazoles to the center of the heme.

In this paper we describe efficient syntheses of protoporphyrin IX analogues bearing 6 and 7 butyric side chains (i.e., one carbon lengthened over the natural heme ligand) and 6 and 7 acetic side chains (i.e., one carbon shortened). These compounds are required for X-ray structural investigations of heme proteins and reconstituted hemes and for studies on the oxygen and carbon binding characteristics of the resulting novel heme proteins. We also describe a modification of the 6,7-dibutyric synthesis to give the 6,7-di-*n*-propyl analogue which was required for clarification of certain problems associated with self-aggregation of magnesium(II) and zinc(II) porphyrins in solution.[3,4]

Synthesis of the Diacetic Porphyrin, 6,7-Bis[(methoxycarbonyl)methyl]-1,3,5,8-tetramethyl-2,4-divinylporphyrin (2). The porphyrin 2 was synthesized directly from monopyrroles via the a,c-biladiene route.[5] Though approaches by degradation of commercial protohemin (3) were considered, we felt that the sensitive vinyl groups in 3 might cause undue problems; moreover, in the

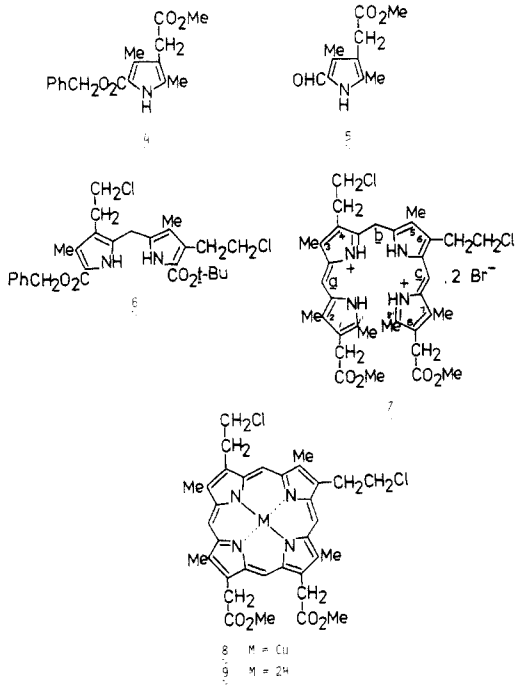
(1) Antonini, A.; Brunori, M. "Hemoglobin and Myoglobin in Their Reactions with Ligands"; North-Holland: Amsterdam, 1977, and references therein.
(2) E.g.: Perutz, M. F. *Br. Med. Bull.* **1975**, *32*, 195–208.
(3) Abraham, R. J.; Fell, S. C. M.; Pearson, H.; Smith, K. M. *Tetrahedron*, **1979**, *35*, 1759–1766.
(4) Abraham, R. J.; Eivazi, F.; Pearson, H.; Smith, K. M. *J. Chem. Soc., Chem. Commun.* **1976**, 698–699, 699–700.
(5) Baptista de Almeida, J. A. P.; Kenner, G. W.; Rimmer, J.; Smith, K. M. *Tetrahedron* **1976**, *32*, 1793–1799.

0022-3263/81/1946-2189$01.25/0



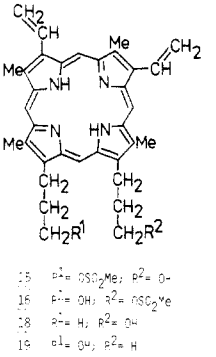
event that a monoacetic monopropionic porphyrin were required, this could be synthesized with only a minor variation in the a,c-biladiene approach.[5]

Thus, the monopyrrole **4** was synthesized from benzyl oximinoacetoacetate and methyl 3-acetyl-4-oxopentanoate, as previously described.[6] Catalytic debenzylation and Vilsmeier formylation gave the 2-formylpyrrole **5**. Two moles of this was condensed with the known dipyrrylmethane **6**[7] in 40% HBr in acetic acid to give a 57% yield of the a,c-biladiene dihydrobromide, **7**. This tetrapyrrole was cyclized, using copper(II) chloride in dimethylformamide, to give the copper(II) porphyrin **8**, which was demetalated in 10% sulfuric acid in trifluoroacetic acid to give the 2,4-bis(2-chloroethyl)porphyrin **9** in 26% yield from the a,c-biladiene **7**. After double dehydrochlorination using aqueous potassium hydroxide in pyridine, a 78% yield of the required porphyrin **2** was obtained.



Synthesis of the Dibutyric Porphyrin, 6,7-Bis[3-(methoxycarbonyl)propyl]-1,3,5,8-tetramethyl-2,4-divinylporphyrin (10). The Grinstein method[8] was used to obtain protoporphyrin IX dimethyl ester from commercial protohemin (**3**). Reduction of the 6,7-propionic ester side chains was readily accomplished with lithium aluminum hydride in tetrahydrofuran, and a 92% yield of the required 6,7-bis(3-hydroxypropyl)porphyrin, **11**, was obtained. With triphenylphosphine and carbon tetrabromide the 6,7-bis(3-bromopropyl)porphyrin **12** was produced, and the carbon chain was lengthened by treatment with sodium cyanide in a two-phase system using a phase-transfer catalyst, giving an 84% yield of the 6,7-bis(3-cyanopropyl)porphyrin **13**. Methanolysis was achieved by using saturated hydrogen chloride in methanol, and a 44% yield of the required porphyrin **10** was obtained.

Synthesis of the Dipropylporphyrin, 1,3,5,8-tetramethyl-6,7-dipropyl-2,4-divinylporphyrin (14). A slight modification of the synthesis used for the dibutyric porphyrin, **10**, was employed for this porphyrin. Thus, the 6,7-bis(3-hydroxypropyl)porphyrin **11** was treated with methanesulfonyl chloride to give a mixture of monomesylates, **15** and **16**, and the dimesylate, **17**. Reduction of the zinc(II) complex of **17** once more with lithium aluminum hydride gave the required 6,7-dipropylporphyrin **14** after removal of the zinc. Likewise, the monomesylate mixture (**15** and **16**) was reduced with lithium aluminum hydride to give the corresponding monohydroxypropyl monopropyl compounds **18** and **19**, which were retreated with methanesulfonyl chloride to give **20** and **21** and then retreated with lithium aluminum hydride to give a second batch of the required dipropylporphyrin **14**.



X-ray and carbon monoxide/oxygen binding studies of the myoglobins and hemoglobins obtained by reconstitution with the hemes derived from the above porphyrins will be reported elsewhere.

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 (1.5 mm); analytical TLC was performed with Merck silica gel 60 F254 precoated sheets (0.2 mm). Electronic absorption spectra were measured with a Cary 17 spectrophotometer (solutions in methylene chloride) and proton NMR spectra were determined either at 200 or 360 MHz with a Nicolet NT-200 or NT-360 spectrometer (solutions usually in deuteriochloroform with tetramethylsilane as internal standard). Mass spectra (direct insertion probe, 70 eV, 50 μA, source temperature ca. 200 °C) were measured with a Finnegan 3200 mass spectrometer. Protohemin was purchased from Man-Win (Washington, D.C.).

2-Formyl-4-[(methoxycarbonyl)methyl]-3,5-dimethylpyrrole (5). Benzyl 4-[(methoxycarbonyl)methyl]-3,5-dimethylpyrrole-2-carboxylate[6] (**4**; 10.0 g) in 150 mL of tetrahydrofuran and 0.1 mL of triethylamine was hydrogenated over 1.0 g of 10% palladized charcoal, at room temperature and atmospheric pressure, until uptake of hydrogen had ceased and the reaction was determined to be complete by analytical TLC. The catalyst was filtered off on Celite and the filtrate was evaporated

(6) Carr, R. P.; Jackson, A. H.; Kenner, G. W.; Sach, G. S. *J. Chem. Soc. C* **1971**, 487–502.
(7) Caveleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1*, **1974**, 1771–1781.
(8) Fuhrhop, J.-H.; Smith, K. M. In "Porphyrins and Metalloporphyrins"; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; p 802.

to dryness to give a yellowish solid (4-[(methoxycarbonyl)-methyl]-3,5-dimethylpyrrole-2-carboxylic acid) which was not characterized but was treated directly with 25 mL of trifluoroacetic acid. After 30 min, 250 mL of methylene chloride and 250 mL of water were added and the organic layer was separated and washed with 250 mL of saturated aqueous sodium bicarbonate and then 250 mL of water. After evaporation of toluene (as a chaser) the residue was dissolved in 50 mL of dry methylene chloride and was then added dropwise to 100 mL of dry methylene chloride containing the Vilsmeier complex prepared by mixing 20 mL of dry phosphoryl chloride with 20 mL of dry dimethylformamide. (This mixture had been cooled in an ice bath until the mixture became orange and viscous, and finally crystallized. The methylene chloride was then added.) After the mixture was heated under reflux for 1 h and cooled, a solution of 300 g of sodium bicarbonate in 1 L of water was added slowly and carefully, and the mixture was then heated under reflux for a further 1 h and then stirred overnight at room temperature. The organic layer was separated, washed with 200 mL of saturated sodium chloride solution and then with 200 mL of water, and evaporated to dryness before evaporation of toluene as a chaser. The residue was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride and then with 2% methanol in methylene chloride). The latter eluates were evaporated to dryness and the residue was crystallized from methylene chloride/hexane to give the formylpyrrole (1.8 g, 31%) as yellowish prisms: mp 127–127.5 °C; NMR δ 2.33 (6 H, s, 3,5-Me), 3.42 (2 H, s, 4-CH_2CO_2Me), 3.73 (3 H, s, OMe), 9.53 (1 H, s, 2-CHO), 10.85 (1 H, br s, NH); mass spectrum, *m/e* (%) 195 (20), 149 (18), 136 (100), 107 (12).

Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.18. Found: C, 61.51; H, 6.71; N, 7.17.

4,6-Bis(2-chloroethyl)-1,8-bis[(methoxycarbonyl)-methyl]-1′,2,3,5,7,8′-hexamethyl-a,c-biladiene Dihydrobromide (7). *tert*-Butyl 5′-[(benzyloxy)carbonyl]-3′,4-bis(2-chloroethyl)-3,4′-dimethyldipyrrylmethane-5-carboxylate[7] (6; 1.35 g) in 75 mL of tetrahydrofuran and 0.1 mL of triethylamine was hydrogenated over 100 mg of 10% palladized charcoal, at room temperature and atmospheric pressure, until uptake of hydrogen ceased and the reaction was determined to be complete by analytical TLC (usually >1 d). The catalyst was filtered off on Celite and the filtrate was evaporated to dryness to give a pale yellow solid [5-*tert*-[(butyloxy)carbonyl]-3′,4-bis(2-chloroethyl)-3,4′-dimethyldipyrrylmethane-5′-carboxylic acid] which was dissolved directly in 5 mL of trifluoroacetic acid and added to a solution of 990 mg of 2-formyl-4-[(methoxycarbonyl)methyl]-3,4-dimethylpyrrole (5) in 20 mL of methanol. This mixture was treated immediately with 5 mL of 40% HBr in acetic acid. After 30 min the biladiene salt began to crystallize as orange material, so 100 mL of ether was added dropwise over 1.5 h to complete the deposition. The orange product was collected by filtration and washed with cold ether to give the a,c-biladiene (1.304 g, 63%) as bright orange crystals: mp >350 °C; NMR δ 1.99, 2.30, 2.35, 2.75 (3 H, 3 H, 6 H, 6 H, each s, 1′,2,3,5,7,8′-Me), 3.25–3.29 (4 H, s, 1,8-CH_2CO_2Me), 3.07–3.12, 3.57–3.62 (each 4 H, each m, 4,6-CH_2CH_2Cl), 3.71 (6 H, s, OMe), 5.16 (2 H, s, b-CH_2), 7.18 (2 H, s, a,c-CH), 13.40, 13.45, 13.56, 13.63 (each 1 H, each br s, NH^+); UV–vis λ_{max} 427 nm (ϵ 43 600), 504 (114 600).

Anal. Calcd for $C_{35}H_{44}Br_2Cl_2N_4O_4$: 51.55; H, 5.44; N, 6.87. Found: C, 51.28; H, 5.42; N, 6.66.

2,4-Bis(2-chloroethyl)-6,7-bis[(methoxycarbonyl)-methyl]-1,3,5,8-tetramethylporphyrin (9). The foregoing a,c-biladiene 7 (923 mg) and 3.5 g of copper(II) chloride were stirred in 25 mL of dimethylformamide at 150 °C for 6 min. The solution was poured into 250 mL of water containing 15 mL of pyridine and then extracted with four 100-mL portions of chloroform. The combined extracts were washed with two 100-mL portions of water and then evaporated with toluene (as a chaser). The residue was treated with 50 mL of 5% sulfuric acid in methanol at 40 °C for 2 h before addition of 250 mL of chloroform and 250 mL of water. The organic layer was separated, washed with saturated aqueous sodium bicarbonate and then 100 mL of water, and evaporated to dryness, using toluene as a chaser. The residue, containing the crude copper(II) porphyrin, was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride); the bright pink eluates were evaporated to dryness, and the residue was stirred with 25 mL of 10% sulfuric acid in trifluoroacetic acid for 30 min (to remove the copper). Saturated aqueous sodium acetate solution (150 mL) was added and the solution was extracted with four 100-mL portions of chloroform. The combined extracts were washed with 100 mL of saturated aqueous sodium bicarbonate solution and then with 100 mL of water and evaporated to dryness with toluene as a chaser. The residue was treated overnight at room temperature with 50 mL of 5% sulfuric acid in methanol before addition of 200 mL of chloroform and 200 mL of water. The organic layer was separated, washed with 100 mL of saturated sodium bicarbonate solution and then with 100 mL of water, and evaporated to dryness with toluene as a chaser. Crystallization of the residue from methylene chloride/hexane gave purple disk-like crystals (205 mg, 26%): mp 240.5–242 °C; NMR δ −3.83 (2 H, br s, NH), 3.60, 3.63, 3.65, 3.67 (each 3 H, each s, 1,3,5,8-Me), 3.79 (6 H, s, OMe), 4.27–4.33, 4.43–4.50 (each 4 H, each m, 2,4-CH_2CH_2Cl), 5.05–5.07 (4 H, m, CH_2CO_2Me), 9.94, 9.97, 10.05, 10.13 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 636 (72), 635 (78), 634 (100), 633 (46), 600 (34), 599 (29), 575 (25), 541 (10), 527 (12); UV–vis λ_{max} 399 nm (ϵ 205 000), 499 (16 000), 532 (9400), 569 (7000), 622 (3800).

Anal. Calcd for $C_{34}H_{36}Cl_2N_4O_4$: C, 64.26; H, 5.71; N, 8.82. Found: C, 64.34; H, 5.80; N, 8.77.

6,7-Bis[(methoxycarbonyl)methyl]-1,3,5,8-tetramethyl-2,4-divinylporphyrin (2). The foregoing porphyrin 9 (225 mg) in 90 mL of pyridine was heated under reflux under an atmosphere of nitrogen for 10 min before 17 mL of water was added. After another 5 min an aqueous solution of 3% sodium hydroxide (20 mL) was added and refluxing was continued under nitrogen for a further 2.5 h. The mixture was treated with 20 mL of 25% acetic acid in water and was then evaporated to near dryness with toluene as a chaser. Addition of 100 mL of water precipitated the crude product which was collected by filtration and washed with two 50-mL portions of water. The porphyrin was dissolved in 50 mL of 5% sulfuric acid in dry methanol and then left overnight at room temperature. Then, 100 mL of chloroform and 100 mL of water were added and the organic layer was separated, washed with 100 mL of saturated sodium bicarbonate solution and 100 mL of water, and evaporated to dryness. The residue was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride) and evaporation and crystallization of the material from the red eluates gave the porphyrin (157 mg, 78%) as purple prisms: mp dec >250 °C; NMR δ −3.68 (2 H, br s, NH), 3.65, 3.66, 3.71, 3.72 (each 3 H, each s, 1,3,5,8-Me, 3.79 (6 H, s, OMe), 5.07 (4 H, s, 6,7-CH_2CO_2Me), 6.18–6.41 (4 H, m, 2,4-CH=CH_2), 8.23–8.32 (2 H, m, 2,4-CH=CH_2), 10.07, 10.08, 10.17, 10.19 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 562 (100), 561 (49), 504 (26), 503 (45), 444 (9), 443 (10), 429 (8); UV–vis λ_{max} 407 nm (ϵ 155 500), 504 (12 700), 538 (9800), 572 (5800), 624 (4000).

Anal. Calcd for $C_{34}H_{34}N_4O_4$: C, 72.58; H, 6.09; N, 9.96. Found: C, 72.48; H, 6.16; N, 9.78.

6,7-Bis(3-hydroxypropyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin (11). Protoporphyrin IX dimethyl ester[8] (500 mg) in 75 mL of dry tetrahydrofuran was treated with a solution of 85 mg of lithium aluminum hydride in 10 mL of dry tetrahydrofuran. The mixture was stirred at 0 °C in an ice bath for 3 h and the reaction was monitored by analytical TLC. Methanol (2% in water, 400 mL) was added and the mixture was extracted with 250 mL of methylene chloride which was then washed with 100 mL of water, dried (Na_2SO_4), and evaporated to dryness to give a residue which was chromatographed on alumina (Brockmann Grade V, elution with methylene chloride). The first band eluted contained protoporphyrin IX dimethyl ester (35 mg, 7%); continued elution with methylene chloride containing 2% methanol gave a deep red eluate which was evaporated to dryness; the residue was crystallized from methylene chloride/hexane to give the porphyrin (430 mg, 95%): mp >300 °C; NMR δ −3.54 (2 H, br s, NH), 3.40, 3.60 (each 6 H, each s, 1,3,5,8-Me), 3.60–3.85 (8 H, m, $CH_2CH_2CH_2OH$, central methylene obscured, 6.20–6.40 (4 H, m, CH=CH_2), 8.23–8.32 (2 H, m, CH=CH_2), 9.84, 9.99, 10.02, 10.10 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 534 (100), 489 (20), 343 (95), 264 (4); UV–vis λ_{max} 408 nm (ϵ 153 000), 503 (11 000), 535 (7700), 568 (5100), 618 (3600). The infrared spectrum confirmed the absence of any C=O grouping.

Anal. Calcd for $C_{34}H_{38}N_4O_2 \cdot 0.5H_2O$: C, 76.37; H, 7.16; N, 10.48. Found: C, 76.37; H, 7.23; N, 10.30.

6,7-Bis(3-bromopropyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin (12). The foregoing porphyrin (673 mg) in 250 mL of methylene chloride (required brief heating to dissolve) was treated with a solution of 5.0 g of carbon tetrabromide and 4.5 g of triphenylphosphine in 50 mL of dry methylene chloride. The mixture was heated under reflux for 10 min, after which time the reaction was determined to be complete by analytical TLC. The mixture was concentrated to 50 mL and cooled in an ice bath until it solidified. The solid was washed several times with small portions of ice-cold methylene chloride and each time a purple solution of the desired porphyrin was decanted from pale yellow-green crystals of triphenylphosphine and its oxide. All of the methylene chloride fractions were combined and evaporated to dryness. The residue was chromatographed on silica gel (elution with methylene chloride and then chloroform). Evaporation of the red eluates, followed by crystallization from methylene chloride/hexane gave the product (360 mg, 44%) as purple needles: mp 232.5–234 °C; NMR δ −3.63 (2 H, br s, NH), 2.82–2.93 (4 H, m, 6,7-$CH_2CH_2CH_2Br$), 3.63, 3.67, 3.73, 3.76 (each 3 H, each s, 1,3,5,8-Me), 4.23–4.28 (8 H, m, 6,7-$CH_2CH_2CH_2Br$), 6.19–6.43 (4 H, m, $CH{=}CH_2$), 8.26–8.38 (2 H, m, $CH{=}CH_2$), 10.12, 10.14, 10.19, 10.26 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 661 (60), 660 (29), 659 (39), 658 (18), 580 (48), 579 (48), 554 (48), 553 (100), 551 (60), 462 (43); UV–vis δ_{max} 402 (ϵ 190 000), 497 (15 600), 535 (12 300), 563 (7700), 621 (5500).

Anal. Calcd for $C_{34}H_{36}Br_2N_4$: C, 61.83; H, 5.49; N, 8.48. Found: C, 61.93; H, 5.57; N, 8.31.

6,7-Bis(3-cyanopropyl)-1,3,5,8-tetramethyl-2,4-divinylporphyrin (13). A mixture of 460 mg of the foregoing porphyrin, 350 mg of hexadecyltributylphosphonium bromide (phase-transfer catalyst), and 350 mg of sodium cyanide in 100 mL of methylene chloride and 500 mL of water was heated under reflux in the dark for 48 h, after which time the reaction was determined to be complete by analytical TLC. The organic phase was separated, washed with 100 mL of water, and then evaporated, using toluene as a chaser. The residue was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride), the appropriate eluates were evaporated, and the residue was crystallized from methylene chloride/hexane to give the product as purple granular crystals (325 mg, 84%): mp dec >270 °C; NMR δ −3.97 (2 H, br s, NH), 2.51–2.59 (4 H, m, 6,7-$CH_2CH_2CH_2CN$), 2.59–2.64, 4.11–4.19 (each 4 H, each m, 6,7-$CH_2CH_2CH_2CN$), 3.55, 3.56, 3.65, 3.66 (each 3 H, each s, 1,3,5,8-Me), 6.18–6.39 (4 H, m, $CH{=}CH_2$), 8.18–8.29 (2 H, m, $CH{=}CH_2$), 9.83, 9.96, 10.07, 10.12 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 552 (100), 499 (31), 484 (26); UV–vis λ_{max} 401 nm (ϵ 180 700), 498 (13 700), 534 (10 900), 569 (6200), 623 (3900).

Anal. Calcd for $C_{36}H_{36}N_6 \cdot 0.5H_2O$: C, 76.98; H, 6.64; N, 14.96. Found: C, 77.24; H, 6.56; N, 14.95.

6,7-Bis[3-(methoxycarbonyl)propyl]-1,3,5,8-tetramethyl-2,4-divinylporphyrin (10). The foregoing porphyrin (295 mg) was allowed to stand in the dark at room temperature for 16 h in 50 mL of saturated dry hydrogen chloride gas in methanol before dilution of the mixture with 50 mL of water and neutralization with aqueous ammonia. Chloroform (100 mL) was then added and the organic phase was separated, washed with 100 mL of water, and evaporated to dryness with toluene as a chaser. Preparative thick-layer chromatography on silica gel (elution with 2% methanol in methylene chloride) gave the product which was crystallized from methylene chloride/hexane to give 145 mg (44%) as purple needles: mp 219–221 °C; NMR δ −3.78 (2 H, br s, NH), 2.59–2.65 (4 H, m, 6,7-$CH_2CH_2CH_2CO_2Me$), 2.72–2.76, 4.07–4.12 (each 4 H, each m, 6,7-$CH_2CH_2CH_2CO_2Me$), 3.58, 3.59, 3.66, 3.67 (each 3 H, each s, 1,3,5,8-Me), 3.73, 3.75 (each 3 H, each s, OMe), 6.15–6.38 (4 H, m, $CH{=}CH_2$), 8.20–8.31 (2 H, m, $CH{=}CH_2$), 10.00, 10.10, 10.11, 10.13 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 618 (100), 533 (31); UV–vis λ_{max} 400 nm (ϵ 155 200), 500 (14 400), 533 (11 600), 570 (6800), 624 (5600).

Anal. Calcd for $C_{38}H_{42}N_4O_4$: C, 73.76; H, 6.84; N, 9.05. Found: C, 73.49; H, 6.81; N, 8.95.

1,3,5,8-Tetramethyl-6,7-dipropyl-2,4-divinylporphyrin (14). The 6,7-bis(3-hydroxypropyl)porphyrin 11 (400 mg) in 50 mL of dry methylene chloride was cooled to 5 °C and stirred during addition of 1.2 mL of dry triethylamine. A solution of 260 mg of methanesulfonyl chloride in 10 mL of dry methylene chloride was then added dropwise while maintaining the temperature of the mixture at 5 °C (ice bath). The mixture was stirred at this temperature for 2.5 h before it was poured into 500 mL of 1 N hydrochloric acid and the organic phase was washed with excess aqueous sodium bicarbonate solution. The organic phase was then washed with 100 mL of water, dried (Na_2SO_4), and evaporated to dryness to give a residue which was evacuated at 3 mmHg overnight. The residue was chromatographed on a alumina (Brockmann Grade III, elution with methylene chloride). First to be eluted was the **dimesylate 17**, which was crystallized from methylene chloride/hexane to give 145 mg (33%): mp >300 °C; NMR (CF_3CO_2H) δ 3.20 (6 H, s, OSO_2Me), 3.74, 3.78, 3.81 (3 H, 6 H, 3 H, each s, 1,3,5,8-Me), 3.80–4.20 (8 H, m, $CH_2CH_2CH_2O$, central methylene obscured), 6.20–6.70 (4 H, m, $CH{=}CH_2$), 8.10–8.50 (2 H, m, $CH{=}CH_2$), 11.30 (4 H, s, meso-H); mass spectrum, *m/e* (%) 498 (100), 279 (40); UV–vis λ_{max} 406 nm (ϵ 134 200), 505 (12 500), 528 (10 500), 574 (6500), 628 (5200). Anal. Calcd for $C_{36}H_{42}N_4O_6S_2$: C, 62.58; H, 6.13; N, 8.11. Found: C, 62.63: H, 5.99: N, 7.98. The second band to be eluted contained the **monomesylates 15 and 16** (crude weight 60 mg) and finally, after changing the elution solvent to 3% methanol in methylene chloride, some starting material 11 (75 mg, 17%) was recovered.

The dimesylate 17 (250 mg) in 15 mL of dry methylene chloride was treated with 150 mg of zinc(II) acetate in 10 mL of methanol. After the mixture was warmed for 10 min and 50 mL of methanol was added, the zinc(II) complex precipitated out and was collected by filtration before washing with cold methanol (265 mg, 97%). This material was dissolved in 35 mL of dry tetrahydrofuran and stirred in an ice bath during addition of 125 mg of lithium aluminum hydride in 10 mL of tetrahydrofuran. After being stirred for 80 min the mixture was treated with a saturated solution (10 mL) of potassium sodium tartrate to destroy excess hydride. It was poured into 200 mL of methylene chloride which was washed with three 100-mL portions of water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on alumina (Brockmann Grade II, elution with 1:1 toluene/methylene chloride), the fast-running band was collected and evaporated, and the residue was crystallized from methylene chloride/hexane to give the zinc(II) complex of 14 (68 mg, 33%): mp >300 °C; NMR (in $CDCl_3$ only, 4 mg in 0.4 mL)[3,4] δ 1.14, 1.17 (each 3 H, each t, CH_2CH_2Me), 1.90–2.30 (4 H, m, CH_2CH_2Me), 3.21, 3.23, 3.26, 3.32 (each 3 H, each s, 1,3,5,8-Me), 3.60–3.75 (4 H, m, CH_2CH_2Me), 5.86, 6.27 (each 2 H, each m, $CH{=}CH_2$), 7.56, 8.14 (each 1 H, each m, $CH{=}CH_2$), 8.89, 8.98, 9.26 (2 H, 1 H, 1 H, each s, meso-H); NMR (in $CDCl_3$ + 1 equiv of pyrrolidine, same solution as above)[3,4] δ 1.26 (6 H, t, CH_2CH_2Me), 2.26 (4 H, m, CH_2CH_2Me), 4.11 (4 H, t, CH_2CH_2Me), 3.61, 3.64, 3.76 (3 H, 3 H, 6 H, each s, 1,3,5,8-Me), 6.47, 6.64 (each 2 H, each m, $CH{=}CH_2$), 8.45 (2 H, m, $CH{=}CH_2$), 10.00, 10.06, 10.14, 10.22 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) (^{64}Zn) 564 (100), 535 (66), 537 (66), 540 (50), 283 (17); UV–vis λ_{max} 408 nm (ϵ 139 500), 536 (6600), 575 (10 300). Anal. Calcd for $C_{34}H_{36}N_4Zn$: C, 72.14; H, 6.41; N, 9.89; Zn, 11.54. Found: C, 72.11; H, 6.48; N, 9.66, Zn, 11.54. The foregoing zinc(II) complex was demetalated as follows. The product (34 mg) was dissolved in 3 mL of trifluoroacetic acid and after standing for 5 min at room temperature it was poured into 100 mL of water and 50 mL of methylene chloride. After the solution was washed with aqueous sodium bicarbonate and water, the organic phase was dried (Na_2SO_4) and evaporated to dryness to give a residue which was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride). The appropriate eluates were evaporated to dryness and the residue was crystallized from methylene chloride/hexane to give the **dipropylporphyrin 14** (25 mg, 82%): mp 185–186 °C; NMR δ −3.50 (2 H, br s, NH), 1.24 (6 H, t, CH_2CH_2Me), 2.26 (4 H, m, CH_2CH_2Me), 3.90 (4 H, m, CH_2CH_2Me), 3.45, 3.48, 3.51 (3 H, 3 H, 6 H, each s, 1,3,5,8-Me), 6.02, 6.35 (each 2 H, each m, $CH{=}CH_2$), 7.93, 8.55 (each 1 H, each m, $CH{=}CH_2$), 9.78, 9.80, 9.88, 9.94 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 502 (100), 487 (21), 473 (50), 252 (30); UV–vis δ_{max} 407 nm (ϵ 170 000), 505 (15 100), 541 (12 000), 576 (8000), 605 (2100), 630 (5400).

Anal. Calcd for $C_{34}H_{38}N_4$: C, 81.23; H, 7.62; N, 11.15. Found: C, 81.39; H, 7.28; N, 11.04.

The crude monomesylates **15** and **16** (60 mg) were also transformed into the dipropylporphyrin 14 as follows. The material (60 mg) in 10 mL of methylene chloride was treated with 100 mg of zinc(II) acetate in 5 mL of methanol. After being briefly

warmed on a hot-water bath the mixture was poured into 100 mL of water and extracted with 100 mL of methylene chloride, and the organic phase was washed with 100 mL of water, dried (Na_2SO), and evaporated to dryness to give a pink residue which was taken up in 10 mL of dry tetrahydrofuran and reduced with 100 mg of lithium aluminum hydride as described above. After a similar workup, the product crystallized from methylene chloride/hexane and was demetalated by addition of trifluoroacetic acid, as described above. Chromatography of the product on alumina (Brockmann Grade III, elution with methylene chloride) followed by evaporation of the red eluates and crystallization from methylene chloride/hexane gave the **mono(hydroxypropyl)monopropyl isomeric mixture 18 and 19** (45 mg, 70%), mp >300 °C. Anal. Calcd for $C_{34}H_{38}N_4O$: C, 78.73; H, 7.38; N, 10.80. Found: C, 78.53; H, 7.10; N, 10.65. This material was retreated with methanesulfonyl chloride, as described above, to give the corresponding **monomesylate–monopropyl isomeric mixture 20 and 21**: mp >300 °C; NMR δ –3.64 (2 H, br s, NH), 1.05 (3 H, t, CH_2CH_2**Me**), 2.64, 2.72 (each 2 H, each m, CH_2CH_2Me and $CH_2CH_2CH_2O$), 2.90 (3 H, s, OSO_2Me), 3.55 3.70 (6 H, 6 H, each s, 1,3,5,8-Me), 3.85, 4.21 (each 2 H, t, CH_2CH_2Me and $CH_2CH_2CH_2O$), 4.48 (2 H, t, $CH_2CH_2CH_2O$), 6.05, 6.30 (each 2 H, m, $CH{=}CH_2$), 8.30, 8.40 (each 1 H, m, $CH{=}CH_2$, 9.83, 9.98, 10.52, 10.75 (each 1 H, each s, meso-H); mass spectrum, *m/e* (%) 580 (100), 534 (20), 516 (35), 445 (58), 429 (30); UV–vis λ_{max} 406 nm (ε 125 100), 505 (11 800), 539 (10 000), 576 (6000), 628 (5000). Anal. Calcd for $C_{35}H_{40}N_4O_3S$: C, 70.43; H, 6.76; N, 9.39. Found: C, 70.58; H, 6.81; N, 9.40. This material was then further reduced with lithium aluminum hydride, as described above, and gave the dipropylporphyrin **14**, identical with the authentic sample described previously.

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Registry No. **2**, 76915-34-9; **4**, 31837-62-4; **5**, 76915-35-0; **6**, 54605-90-2; **7**, 76915-36-1; **8**, 76916-46-6; **9**, 76915-37-2; **10**, 76915-38-3; **11**, 76915-39-4; **12**, 76915-40-7; **13**, 76915-41-8; **14**, 76915-42-9; **14** Zn(II) complex, 61593-94-0; **15**, 76915-43-0; **16**, 76915-44-1; **17**, 76915-45-2; **18**, 76915-46-3; **19**, 76915-47-4; **20**, 76915-48-5; **21**, 76915-49-6; [(5-*tert*-butyloxy)carbonyl]-3′,4-bis(2-chloroethyl)-3,4′-dimethyldipyrrylmethane-5′-carboxylic acid, 76915-50-9; protoporphyrin IX dimethyl ester, 5522-66-7; 4-[(methoxycarbonyl)-methyl]-3,5-dimethylpyrrole-2-carboxylic acid, 76915-51-0; **17** zinc(II) complex, 76916-47-7.

Synthesis of *N*-Nitrosocimetidine Hydrate and Nitrate and Tritium-Labeling Studies

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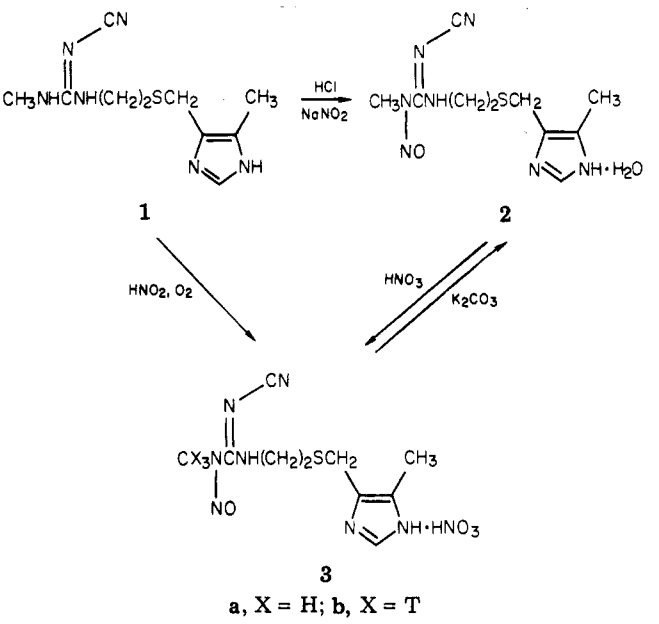
N-Nitrosamines have recently received much attention[1] since the discovery of their carcinogenic and mutagenic properties.[2] The ready availability of their precursors, nitrite and secondary amines, in the environment and in foodstuffs has prompted many investigators to study the kinetics of nitrosamine formation[3] and to develop various techniques for their quantitative determination.[4] Our interest in investigating the metabolic fate of nitrosamines[5] both in vivo and in vitro led us to prepare both cold (unlabeled) and hot (labeled) nitrosamines.

Scheme I



Cimetidine is a highly successful commercial anti-ulcer agent used widely both in Europe and the U.S. Cimetidine (**1**) is a secondary amine and therefore is potentially N-nitrosated readily under pH conditions existing in the stomach of humans (Scheme I).

N-Nitrosocimetidine monohydrate (**2**), mp 23–28 °C, was prepared in 85% yield by nitrosating cimetidine (**1**) with 3 equiv of sodium nitrite for 1 h at 0 °C in the presence of excess hydrochloric acid, followed by basification to pH 10 and extraction with ethyl acetate.

The ^1H NMR spectrum of **1** in Me_2SO-d_6 shows, among other signals, a resonance at δ 2.70 (d) for the methyl group, CH_3NH, of cimetidine which coalesces to a sharp singlet at δ 3.20 upon nitrosation as a consequence of nitrosation of the nitrogen adjacent to this methyl group. On the other hand, nitrosation for a period of 2 h at 0 °C with 5 equiv of sodium nitrite in an open system with atmospheric oxygen freely available results in the separation of nitrosocimetidine nitrate (**3a**) as a yellow solid, mp 143–144 °C (from ethanol),[6] in 75% yield. Its ^1H NMR spectrum matches that of **2**. Compounds **2** and **3a** could be interconverted. Addition of concentrated nitric acid to a solution of **2** in ether–ethanol (1:1) resulted in separation of **3a** and basification of an aqueous solution of **3a** with solid potassium carbonate caused separation of **2** as a yellow oil which solidified upon cooling.

The preparation of tritium-labeled nitrosocimetidine nitrate (**3b**) was undertaken for metabolic and animal carcinogenicity studies. The nitrate salt is convenient to work with as it is soluble in water. Labeled **3** had 83.1% retention of radioactivity and it was isolated in an overall yield of 75%.

Cimetidine and *N*-nitrosocimetidine were studied by differential pulse polarography (DPP).[7] Stock solutions were prepared in neutral water. The polarograms were

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