Normal and Abnormal Heme Biosynthesis. 2.[1] Synthesis and Metabolism of Type-III Pentacarboxylic Porphyrinogens: Further Experimental Evidence for the Enzymic Clockwise Decarboxylation of Uroporphyrinogen-III

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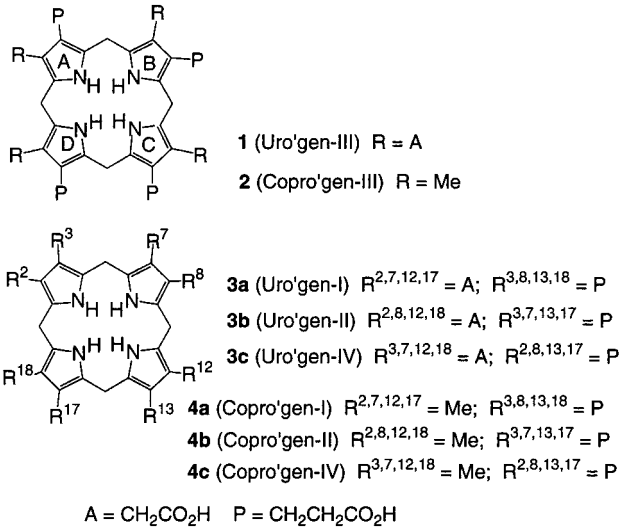
Uroporphyrinogen decarboxylase catalyses the sequential decarboxylation of uroporphyrinogen-III (**1**) to give coproporphyrinogen-III (**2**), a precursor to the hemes and chlorophylls. This involves the decarboxylation of four nonequivalent acetate side chains to produce methyl units and in principle could take place by 24 different pathways involving up to 14 intermediary porphyrinogens. In the past, seemingly contradictory data have been presented that either support an ordered "clockwise" decarboxylation pathway or a random decarboxylation process. Four pentacarboxylate porphyrinogens might be involved immediately before the formation of **2**, and these compounds have been synthesized as the corresponding porphyrin pentamethyl esters via tripyrrene and *a*,*c*-biladiene intermediates. Hydrolysis of the methyl esters and reduction with 3% sodium amalgam gave the required porphyrinogens, and these were incubated with crude enzyme preparations derived from chicken red cell hemolysates. One of these pentacarboxylate porphyrinogens (**5dab**) consistently proved to be a much better substrate than the other three, providing new support for the "clockwise" pathway for coproporphyrinogen-III formation.

Introduction

Uroporphyrinogen decarboxylase (UPD; EC 4.1.1.37) catalyses the conversion of uroporphyrinogen-III (uro'gen-III; **1**) to coproporphyrinogen-III (copro'gen-III; **2**), whereby four acetate side chains are decarboxylated to give methyl units (Chart 1).[2] This is the first enzymic step in the heme and chlorophyll biosynthetic pathways to occur solely at the tetrapyrrolic macrocycle level and represents the initial branch point from corrin/vitamin B_{12} biosynthesis.[3] UPD has a low substrate specificity and also converts the type isomers uro'gens I, II, and IV (**3a**−**c**) to the corresponding copro'gens **4a**−**c** (Chart 1).[4] The mechanism for this reaction is believed to involve initial protonation onto the pyrrole nucleus, which facilitates a deprotonation−decarboxylation (Scheme 1). Subsequent protonation onto the resulting exocyclic methylene, followed by a tautomerization, affords the methyl-substituted pyrrole.[5] This process has been shown to occur with retention of configuration using stereospecifically doubly labeled $^2H/^3H$ succinate,[6] implying that the chemistry occurs in a highly ordered "micro-environment".[5] UPD has been isolated and fully or partially purified from several sources, including human[7,8] and chicken erythrocytes,[9] bovine liver,[10] tobacco leaves,[11] the yeast *Saccharomyces cerevisiae*,[12] and the bacteria *Rhodobacter palustrus*[13] and *Rhodobacter sphaeroides*.[14] The enzyme is sensitive to polychlorionated hydrocarbons[15] and is inhibited by heavy metals[16] and sulfhydryl reagents,[7] indicating the presence of an essential cysteine residue

Chart 1. Uro'gen- and Copro'gen-Type Isomers



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(1) Part 1: Lash, T. D.; Mani, U. N.; Drinan, M. A.; Zhen, C.; Hall, T.; Jones, M. A. *J. Org. Chem.* **1999**, *64*, 464−477.

(2) *Biosynthesis of Tetrapyrroles*; Jordan, P. M., Ed.; Elsevier: London, 1991.

(3) Scott, A. I. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1223. Battersby, A. R. *Science* **1994**, *264*, 1551. Battersby, A. R.; Leeper, F. J. In *Topics in Current Chemistry 195. Biosynthesis: Polyketides and Vitamins*; Leeper, F. J., Vederas, J. C., Eds.; Springer-Verlag: Berlin, 1998; pp 143−193.

(4) Smith, A. G.; Francis, J. E. *Biochem. J.* **1979**, *183*, 455.

(5) Akhtar, M. In *The Biosynthesis of Tetrapyrrolic Pigments*; Wiley: Ciba Foundation Symposium 180; Chichester, 1994; pp 130−155.

(6) Barnard, G.; Akhtar, M. *J. Chem. Soc., Chem. Commun.* **1975**, 494.

(7) De Verneuil, H.; Sassa, S.; Kappas, A. *J. Biol. Chem.* **1983**, *258*, 2454. Elder, G. H.; Tovey, J. A.; Sheppard, D. M. *Biochem. J.* **1983**, *215*, 45.

(8) Mukerji, S. K.; Pimstone, N. R. *Int. J. Biochem.* **1992**, *24*, 105.

(9) Kawanishi, S.; Seki, Y.; Sano, S. *J. Biol. Chem.* **1983**, *258*, 4285.

(10) Straka, J. G.; Kushner, J. P. *Biochemistry* **1983**, *22*, 4664.

(11) Chen, T. C.; Miller, G. W. *Plant Cell Physiol.* **1974**, *15*, 993.

(12) Felix, F.; Brouillet, N. *Eur. J. Biochem.* **1990**, *188*, 393.

(13) Koopman, G. E.; Juknat de Gweralnik, A. A.; Battle, A. M. del C. *Int. J. Biochem.* **1986**, *18*, 935.

(14) Jones, R. M.; Jordan, P. M. *Biochem. J.* **1993**, *293*, 703.

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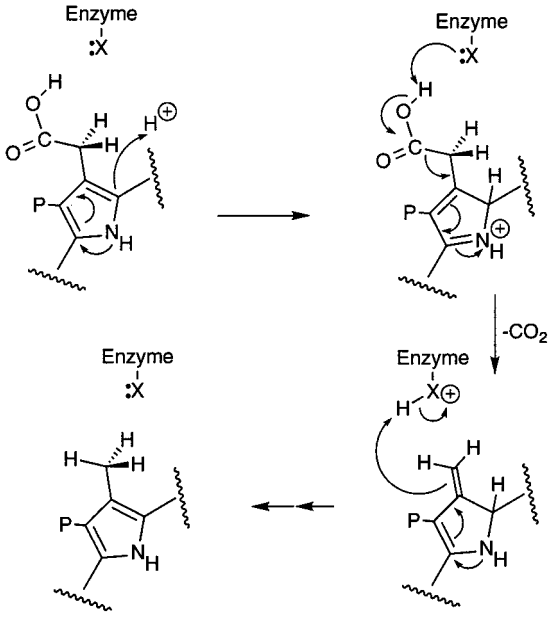
Scheme 1. Proposed Mechanism for the Enzymic Decarboxylation of the Acetate Side Chains of Uroporphyrinogen-III

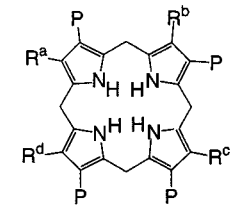


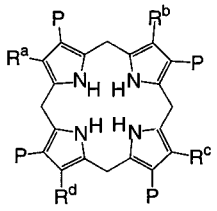
at the active site. In humans, defects in hepatic UPD give rise to the disease porphyria cutanea tarda (PCT),[17,18] which is associated with skin photosensitivity and liver damage. Hepatic porphyrias may be induced by polyhalogenated aromatic hydrocarbons,[19,20] including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin,[21] and can also result from heavy metal poisoning[22] and acute alcoholism.[23] An underlying association of PCT with human immunodeficiency viral (HIV) infection has also been reported.[24]

Clockwise Decarboxylation Hypothesis

UPD is known to sequentially decarboxylate the four acetate residues of uro'gen-III via hepta-, hexa-, and pentacarboxylate intermediates **5**−**7** (Chart 2).[25,26] These species, usually observed after conversion to the corresponding porphyrins, have been isolated and identified from several natural sources and in enzyme incubation studies.[25−27] In addition, relatively large amounts of these porphyrins are excreted in the urine and feces of patients suffering from PCT[26,28] and rats poisoned with hexachlorobenzene (HCB) (the latter represents an animal model for PCT).[26] Due to the asymmetry of uro'gen-III, the first decarboxylation can occur, in principle, at four different sites to produce four heptacarboxylate porphyrinogens **7a**−**d** (Scheme 2; Chart 2).[26] Each "hepta" could be decarboxylated in three ways to give a total of 6 hexacarboxylate porphyrinogens **6ab**, **6ac**, **6da**, **6bc**, **6bd**, and **6cd**, and these "hexas" can each be converted in two ways to produce four different pentacarboxylate porphyrinogens **5dab**, **5abc**, **5acd**, and **5bcd**, which in turn would all produce copro'gen-III.[26] Hence, there are 4! or 24 possible pathways between **1** and **2** (Scheme 2) that could involve a total of 14 different intermediates (Chart 2). Enzyme-catalyzed reactions are nearly always highly regio- and stereoselective, and only one of these pathways might be expected to be favored under physiological conditions. In a monumental and groundbreaking endeavor, Jackson et al. isolated the hepta-, hexa-, and pentacarboxylate porphyrins from the feces and urine of HCB poisoned rats and compared these materials by europium shift reagent proton NMR spectroscopy to synthetic samples of all 14 of the possible intermediates in the form of the corresponding porphyrin methyl esters.[26,28,29] These results clearly demonstrated that

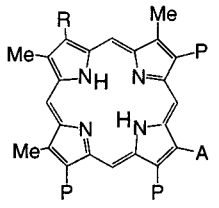
Chart 2. Type-III and Type-I Intermediates and Abnormal Metabolites





7d R^d = Me; $R^{a,b,c}$ = A
7a R^a = Me; $R^{a,b,c}$ = A
7b R^b = Me; $R^{a,c,d}$ = A
7c R^c = Me; $R^{a,b,d}$ = A
6da $R^{d,a}$ = Me; $R^{b,c}$ = A
6ab $R^{a,b}$ = Me; $R^{c,d}$ = A
6ac $R^{a,c}$ = Me; $R^{b,d}$ = A
6bc $R^{b,c}$ = Me; $R^{a,d}$ = A
6bd $R^{b,d}$ = Me; $R^{a,c}$ = A
6cd $R^{c,d}$ = Me; $R^{a,b}$ = A
5dab $R^{d,a,b}$ = Me; R^c = A
5abc $R^{a,b,c}$ = Me; R^d = A
5acd $R^{a,c,d}$ = Me; R^b = A
5bcd $R^{b,c,d}$ = Me; R^a = A

7-I R^a = Me; $R^{b,c,d}$ = A
6-Iab $R^{a,b}$ = Me; $R^{c,d}$ = A
6-Iac $R^{a,c}$ = Me; $R^{b,d}$ = A
5-I $R^{a,b,c}$ = Me; R^d = A

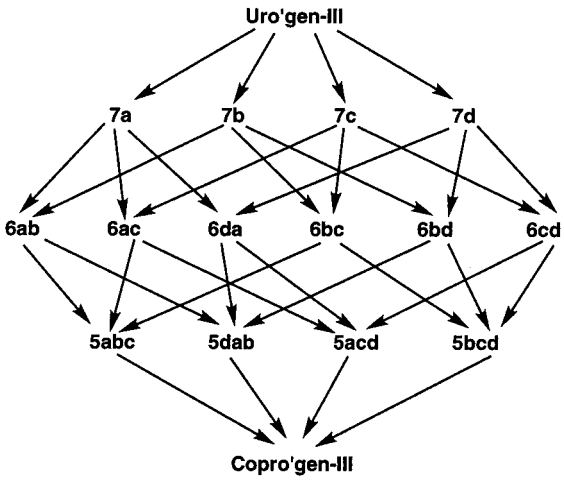


8
"Isocoproporphyrins"
a. R = Et; b. R = V;
c. R = H; d. R = CH(OH)CH_3;
e. R = $COCH_3$

A = CH_2CO_2H P = $CH_2CH_2CO_2H$

(15) De Verneuil, H.; Sassa, S.; Kappas, A. *Biochem. J.* **1983**, *214*, 145. Kawanishi, S.; Seki, Y.; Sano, S. *J. Biol. Chem.* **1983**, *258*, 4285.
(16) Kushner, J. P.; Lee, G. R.; Nacht, S. *J. Clin. Invest.* **1972**, *51*, 3044.
(17) Elder, G. H. *Semin. Liver Dis.* **1998**, *18*, 67.
(18) Moore, M. R. *Int. J. Biochem.* **1993**, *25*, 1353. McDonagh, A. F.; Bissell, D. M. *Semin. Liver Dis.* **1998**, *18*, 3.
(19) Strik, J. J. W. T. A.; Koeman, J. H. *Chemical Porphyria in Man*; Elsevier: Amsterdam, 1979.
(20) Elder, G. H. In *Heme and Hemoproteins*; De Matteis, F., Aldridge, W. N., Eds.; Springer-Verlag: Berlin, 1978; pp 157−200. Sinclair, P. R.; Elder, G. H.; Bement, W. J.; Smith, S. G.; Bonkowsky, H. L.; Sinclair, J. F. *FEBS Lett.* **1983**, *152*, 217. Swain, M. G.; Follows, S. B.; Marks, G. S. *Can. J. Physiol. Pharmacol.* **1983**, *61*, 105.
(21) Goldstein, J. A.; Links, P.; Bergman, H. *Biochem. Pharmacol.* **1982**, *31*, 1607.
(22) Woods, J. S.; Kardish, R.; Fowler, B. A. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 264.
(23) McColl, K. E. L.; Thompson, G. G.; Moore, M. R.; Goldberg, A. *Eur. J. Clin. Invest.* **1980**, *10*, 107.
(24) Wissel, P. S.; Sordillo, P.; Anderson, K.; Sassa, S.; Savillo, R. L.; Kappas, A. *Am. J. Hemat.* **1987**, *25*, 107. Cohen, P. R. *J. Acq. Immune Def. Synth.* **1991**, *4*, 1112. Conlan, M. G.; Hoots, W. K. *J. Am. Acad. Dermat.* **1992**, *26*, 857.
(25) Mauzerall, D.; Granick, S. *J. Biol. Chem.* **1958**, *232*, 1141. Battle, A. M. del C.; Grinstein, M. *Biochim. Biophys. Acta* **1964**, *82*, 1. San Martin de Viale, L. C.; Grinstein, M. *Biochim. Biophys. Acta* **1968**, *158*, 79. Frydman, R. B.; Tomaro, M. L.; Wanschelbaum, A.; Frydman, B. *FEBS Lett.* **1972**, *26*, 203. Smith, S. G. *Br. J. Dermat.* **1975**, *93*, 291.
(26) Jackson, A. H.; Sancovich, H. A.; Ferramola, A. M.; Evans, E.; Games, D. E.; Matlin, S. A.; Elder, G. E.; Smith, S. G. *Philos. Trans. R. Soc. London B* **1976**, *273*, 191. The number/letter codes for **5**−**7**, which were proposed in this paper, indicate the total number carboxylate side chains and which acetate units have been decarboxylated (relative to uro'gen-III). For instance, **5dab** is a pentacarboxylate porphyrinogen that has lost the acetate moieties on rings D, A, and B but retains this unit on ring C.
(27) Battersby, A. R.; Hunt, E.; McDonald, E.; Paine, J. B., III; Saunders, J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1008.

Scheme 2. Random Decarboxylation of Uro'gen-III



single hepta-, hexa-, and pentacarboxylate isomers predominate and these corresponded to **7d**, **6da**, and **5dab**. A sample of a heptacarboxylate porphyrin was also isolated from a PCT patient and shown to be indistinguishable from **7d**.[28,31] Furthermore, in concurrent investigations, Battersby and co-workers isolated a heptacarboxylate porphyrin from incubations of porphobilinogen (PBG, a precursor to uro'gen-III[1]) with avian red cells containing high concentrations of NaCl and again demonstrated that this metabolic product corresponded to **7d**.[27] These results suggested that the decarboxylation of uro'gen-III occurs via a preferred pathway starting at ring D and proceeds in a clockwise fashion to ring A, then B, and finally ring C (Scheme 3).[26,32] Further evidence for this pathway comes from the isolation of a group of abnormal metabolites known as the isocoproporphyrins (Chart 2; **8a**−**e**), which have been isolated from porphyric patients and HCB poisoned rats.[33,34] These are believed to arise from **5dab** being acted upon out of sequence by the next enzyme in the heme pathway, coproporphyrinogen oxidase,[1] after it has escaped from UPD.[35,36] Porphyrin **8b** (dehydroisocoproporphyrin) would be formed from oxidation of the resulting porphyrinogen, while **8a** and **8c**−**e** presumably result from bacterial degradation in the intestinal tract. Under abnormal conditions dehydroisocopro'gen may be involved in an alternative, albeit less efficient, route for heme biosynthesis.[35−37] Uro'gen-I (**3a**) is generated in substantial quantities in congenital erythropoietic porphyria and is metabolized by UPD to give copro'gen-I (**4a**).[38] Only one hepta- (**7-I**) and one pentacarboxylate (**5-I**) intermediate are possible in this case, although two isomeric hexacarboxylate species **6-Iab** and **6-Iac** (Chart 2) might arise (i.e., there are two possible metabolic pathways).[39] HPLC analyses and total synthesis were used to conclusively demonstrate the presence of both type-I hexacarboxylate isomers in samples derived from human and bovine porphyrics as well as for biochemical studies with chicken red cell hemolysates, and thus, it was concluded that the decarboxylation of uro'gen-I occurs by "a nonspecific route".[39]

However, the data for the type-III series are far from clear-cut. Analysis of the porphyrins present in normal human urine showed the presence of mixed type-III pentacarboxylate isomers,[40] but these were assumed to be minor metabolites that resulted from leakage from this relatively "promiscuous" enzyme.[5,40] However, more detailed chromatographic investigations by Lim and coworkers showed[41] that mixed hepta-, hexa-, and pentacarboxylate fractions were present in normal and PCT urine, although the "hepta" fraction from PCT did indeed appear to be primarily **7d**,[42] and this group concluded that the enzymic decarboxylation of uro'gen-III does not always proceed in a clockwise manner.[41,42] More damning evidence came from enzymic studies, initially using chicken red cell hemolysates (CRH).[32,43] Incubations of uro'gen-III with CRH were shown to produce at least two heptacarboxylate isomers, while **7d** gave at least two hexacarboxylate porphyrins (three are possible) and three different pentacarboxylate porphyrins.[32,43] **6da**, **6bd**, and **6cd**, the hexacarboxylate porphyrinogens that might arise from **7d**, all gave mixtures of two pentacarboxylate metabolites on incubation with CRH.[43] Subsequently, Luo and Lim demonstrated that incubations of uro'gen-III with erythocyte and hepatic human UPD gave all 14 of the possible intermediates (Chart 2),[42] while Jones and Jordan showed that UPD derived from *Rh. sphaeroides* also produced random mixtures of heptacarboxylate metabolites.[14] Luo and Lim concluded that "the normal decarboxylation pathway is random in nature" and suggested that **7d** only predominated under abnormal circumstances.[42] While these data seem to support such a statement, the observations of single isomers in the feces and urine from HCB poisoned rats and PCT,[26] as well as from incubations of PBG with highly saline preparations of CRH,[27] were difficult to reconcile with this hypothesis. It is not really plausible that abnormal conditions would result in a preferred pathway while normal metabolism of uro'gen-III by UPD is random. The putative intermediates **7d**, **6da**, and **5dab** are all excellent substrates for UPD,[26,28,43,44] and while all 14 possible intermediates are metabolized by UPD not all of them are good substrates, so this phenomenom cannot be ascribed to the accumulation of the poorer substrates

(28) Jackson, A. H.; Ferramola, A. M.; Sancovich, H. A.; Evans, E.; Matlin, S. A.; Ryder, D. J.; Smith, S. G. *Ann. Clin. Res.* **1976**, *8*, Suppl. 17, 64.

(29) Jackson, A. H.; Sancovich, H. A.; Ferramola de Sancovich, A. M. *Bioorg. Chem.* **1980**, *9*, 71. See also: Chakrabarty, M.; Ali, S. A.; Philip, G.; Jackson, A. H. *Heterocycles* **1981**, *15*, 1199.

(30) Jackson, A. H. *Seminar Hematol.* **1977**, *14*, 193.

(31) Ryder, D. J. Ph.D. Dissertation, University of Wales, College of Cardiff, 1977.

(32) Lash, T. D. *Biochem. J.* **1991**, *278*, 901.

(33) Elder, G. H. *Biochem. J.* **1972**, *126*, 877. Elder, G. H. *J. Clin. Pathol.* **1975**, *28*, 601. Stoll, M. S.; Elder, G. H.; Games, D. E.; O'Hanlon, P.; Millington, D. S.; Jackson, A. H. *Biochem. J.* **1973**, *131*, 429. Elder, G. H. *Seminar Hematol.* **1977**, *14*, 227. Smith, S. G. *Biochem. Soc. Trans.* **1977**, *5*, 1472. Luo, J.; Lim, C. K. *Biomed. Chromatogr.* **1995**, *9*, 113.

(34) (a) Jackson, A. H.; Lash, T. D.; Ryder, D. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 287. (b) Clezy, P. S.; Hai, T. T. *Aust. J. Chem.* **1976**, *29*, 1561.

(35) Elder, G. H.; Evans, J. O. *Biochem. J.* **1978**, *169*, 205.

(36) Jackson, A. H.; Lash, T. D.; Ryder, D. J.; Smith, S. G. *Int. J. Biochem.* **1980**, *12*, 775.

(37) Kurlandzka, A.; Zoladek, T.; Rytka, J.; Labbe-Bois, R. *Biochem. J.* **1991**, *273*, 246.

(38) Desnick, R. J.; Glass, I. A.; Xu, W.; Solis, C.; Astrin, K. H. *Semin. Liver Dis.* **1998**, *18*, 77.

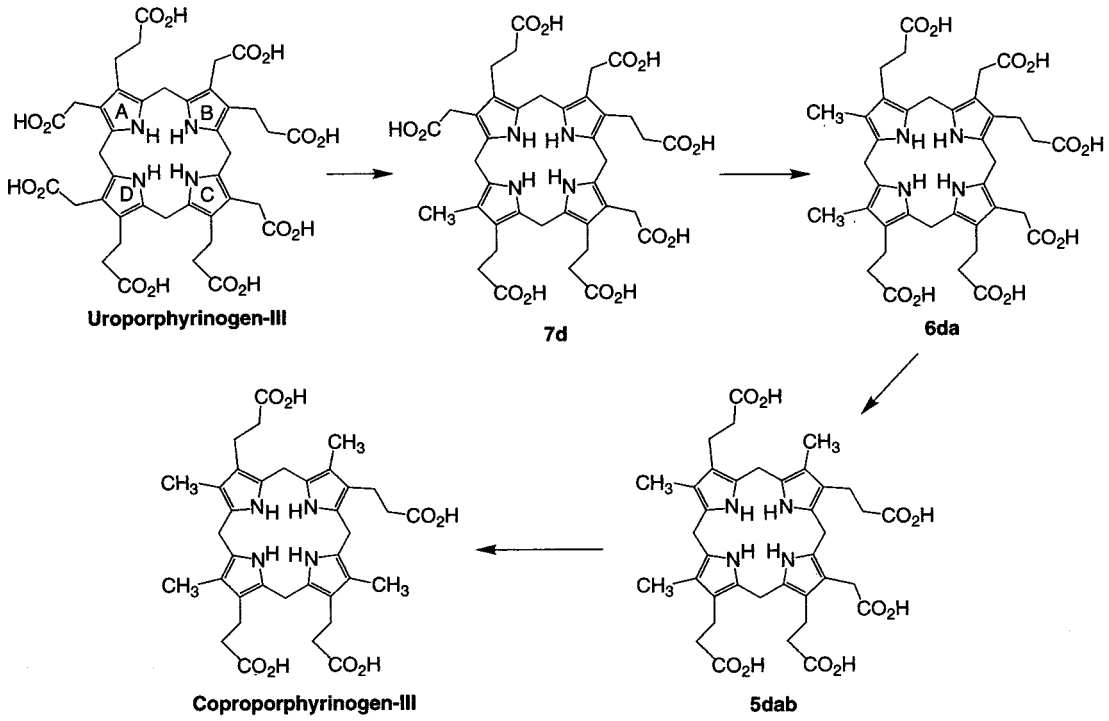
(39) Jackson, A. H.; Rao, K. R. N.; Supphayen, D. M.; Smith, S. G. *J. Chem. Soc., Chem. Commun.* **1977**, 696. Jackson, A. H.; Supphayen, D. M. *J. Chem. Soc., Perkin Trans. 1* **1987**, 277.

(40) Smith, S. G.; Rao, K. R. N.; Jackson, A. H. *Int. J. Biochem.* **1980**, *12*, 1081.

(41) Lim, C. K.; Rideout, J. M. *J. Liquid Chromatogr.* **1983**, *6*, 1969. Lim, C. K.; Rideout, J. M.; Wright, D. J. *J. Chromatogr.* **1983**, *282*, 629. Li, F.; Lim, C. K.; Peters, T. J. *Biochem. J.* **1987**, *243*, 621. Lim, C. K.; Li, F.; Peters, T. J. *Biochem. J.* **1987**, *247*, 229.

(42) (a) Luo, J.; Lim, C. K. *Biochem. J.* **1990**, *268*, 513; (b) *J. Chromatogr.* **1991**, *566*, 409.

Scheme 3. "Clockwise" Decarboxylation of Uroporphyrinogen-III



under abnormal or pathological conditions. One possibility is that the previous enzyme in the heme pathway, uro'gen-III synthetase, hands on the substrate to UPD in a specific orientation, and under these circumstances selective "clockwise" decarboxylation takes place.[32] On the other hand, when UPD is presented with uro'gen-III or a later intermediate, the substrate may associate with the active site(s)[45] in a random orientation, and this leads to isomeric mixtures of metabolites. The ability of the enzyme to accept porphyrinogens randomly may reflect the need to process and excrete type-I isomers that may arise even in normal metabolism. This proposal implies that uro'gen-III undergoes a selective clockwise decarboxylation under physiological conditions and the products observed in urine are minor abnormal metabolites that have escaped from the binding pocket. Further, biosynthetic precursors to uro'gen-III, such as PBG, would be expected to produce single type-III hepta-, hexa-, and pentacarboxylate porphyrinogens in biochemical studies using mixed enzyme systems.[32] To test this postulate, Luo and Lim recently carried out incubations of PBG with crude enzyme preparations from human erythrocytes and analyzed the resulting products by reversed phase HPLC.[46] These data showed that **7d**, **6da**, and **5dab** were virtually the only detectable products, lending support to the proposal. However, incubations of very low concentrations of radiolabeled uro'gen-III with UPD also appeared to show an increased selectivity for the "clockwise" pathway, and this may suggest that the enzyme selectivity is in actual fact concentration dependent.[46]

An orderly "clockwise" decarboxylation pathway is likely to be prevalent under normal physiological conditions, and this should result in UPD having a preference for porphyrinogens **7d**, **6da**, and **5dab** over the other 11 possible intermediates between **1** and **2** (Chart 2). Due to the relative ease of synthesis, and their potential involvement in alternative biosynthetic pathways,[35−37] the four type-III pentacarboxylate porphyrinogens were selected for further study. Although all four of these isomers have been previously shown to be metabolized by CRH, the kinetic studies were based on minimal data (in some cases only two data points were used), and it is difficult to draw conclusions from these results.[28,44] While **5bcd** was reported to be a poor substrate, the other three isomers were apparently easily metabolized by UPD.[44] We anticipated that more subtle distinctions could be discerned by analysis of more detailed kinetic studies.[47]

Synthesis of Type-III Pentacarboxylate Porphyrins

The kinetic studies required synthetic samples of **5dab**, **5abc**, **5acd**, and **5bcd**, and these were prepared as the corresponding porphyrin pentamethyl esters. These compounds were previously synthesized by Jackson et al.[29] and Clezy et al.[48] using a combination of the MacDonald[49] and *b*-oxobilane routes.[50] In addition, the porphyrin corresponding to **5dab** was prepared by the copper(II) chloride mediated cyclization of an *a*,*c*-biladiene.[51] In this work, we wanted to minimize the number

(43) Lash, T. D. Ph.D. Dissertation, University of Wales, College of Cardiff, 1979.

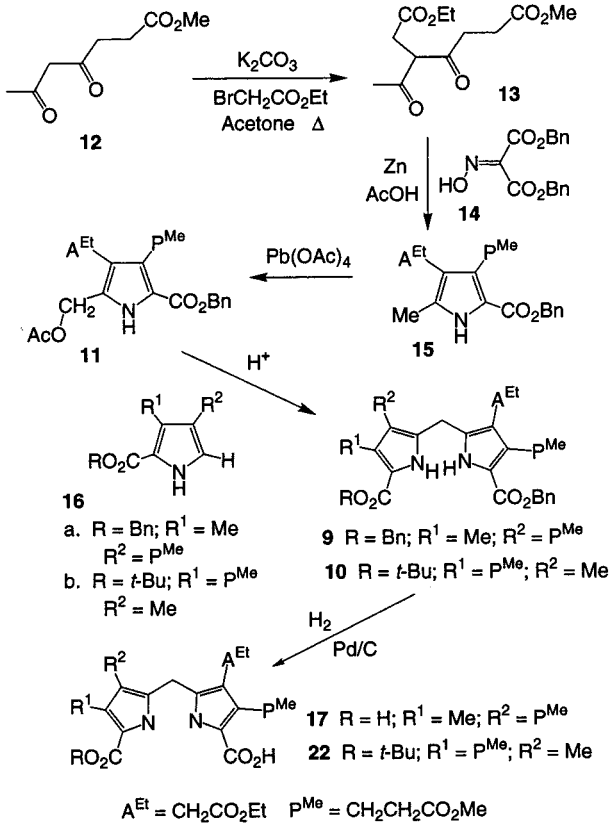
(44) Smith, S. G.; Ferramola, A. M.; Sancovich, H. A.; Evans, E.; Matlin, S. A.; Ryder, D. J.; Jackson, A. H. *Ann. Clin. Res.* **1976**, *8*, Suppl. 17, 89.

(45) Evidence has been presented for the presence of more than one active site in UPD: Cornford, P. *Biochem. J.* **1964**, *91*, 64. Tomio, J.; Garcia, R.; San Martin de Viale, L.; Grinstein, M. *Biochim. Biophys. Acta* **1970**, *198*, 353. Garcia, R. C.; San Martin de Viale, L. C.; Tomio, J. M.; Grinstein, M. *Biochim. Biophys. Acta* **1973**, *309*, 203. Rasmussen, G. L.; Kusner, J. P. *J. Lab. Clin. Med.* **1979**, *93*, 54. Smith, A. G.; Francis, J. E. *Biochem. J.* **1979**, *183*, 455. De Verneuill, H.; Grandchamp, G.; Nordmann, Y. *Biochim. Biophys. Acta* **1980**, *611*, 174. Straka, J. G.; Kushner, J. P.; Burnham, B. F. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1980**, *39*, 2005.

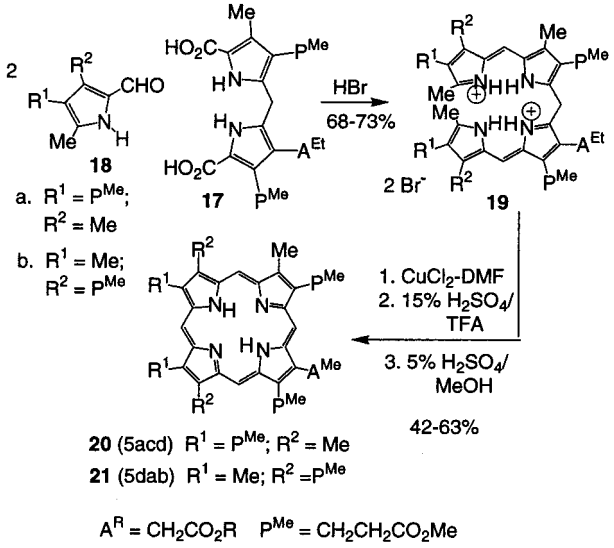
(46) Luo, J.; Lim, C. K. *Biochem. J.* **1993**, *289*, 529.

(47) Preliminary kinetic results for type-III pentacarboxylate porphyrinogens were presented at the 209th National ACS Meeting, Anaheim, CA, April 1995 (Abstract: Lash, T. D.; Mani, U. N.; Lyons, E. A.; Thientanavanich, P.; Hall, T.; Jones, M. A. *Book of Abstracts*, ORGN 344).

Scheme 4



Scheme 5



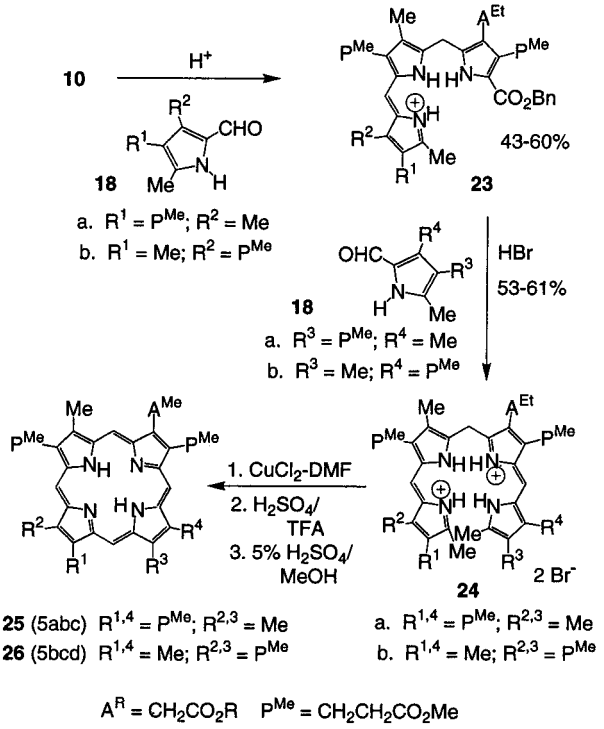
of synthetic intermediates used to prepare all four isomers and selected the dipyrrylmethanes **9** and **10**, both of which can be generated from a common pyrrole **11** bearing an acetate side chain, as the key precursors (Scheme 4). Condensation of dione **12** with ethyl bromoacetate in the presence of potassium carbonate in refluxing acetone gave the diester **13** in moderate yield, and subsequent Knorr−Kleinspehn condensation[52,53] with dibenzyl oximinomalonate (**14**) in the presence of zinc dust and sodium acetate in acetic acid gave the mixed triester **15** in 39% yield. The required (acetoxymethyl)-pyrrole **11** was obtained by the regioselective oxidation of **15** with lead tetraacetate in acetic acid. Montmorillonite clay catalyzed condensation[54] of **11** with α-unsubstituted pyrroles **16a**[55] or **16b**[56] in dichloromethane gave the pivotal intermediates **9** and **10** as white powders in excellent yields.

Although dipyrroles **9** and **10** could be taken on to the targeted pentacarboxylate porphyrin by a number of established methods, the *a,c*-biladiene route[51,57] was selected on the basis of past experience[58] and the availability of the other pyrrolic precursors. Hydrogenolysis of dibenzyl ester **9** over 10% palladium on activated carbon gave the related dicarboxylic acid **17**. Subsequent decarboxylation by treatment with TFA, followed by condensation with 2 equiv of pyrrole aldehyde **18a** or **18b** in the presence of HBr and precipitation with ether, gave the tetrapyrrolic *a,c*-biladiene dihydrobromide salts **19** as red powders (Scheme 5). Cyclization with $CuCl_2$ in DMF at room temperature,[59] followed by demetalation with 15% sulfuric acid−TFA and reesterification with 5% H_2SO_4−methanol, gave the porphyrin pentamethyl esters **20** (precursor to **5acd**) and **21** (precursor to **5dab**) in 42% and 61% yields, respectively (Scheme 5).

The remaining pentacarboxylate porphyrin isomers were prepared from dipyrrole **10**, but this required the use of a more stepwise approach using tripyrrene intermediates.[51] Initially, **10** was hydrogenolyzed over Pd/C to give carboxylic acid **22** (Scheme 4), and attempts were made to condense the monodeprotected dipyrrylmethane with pyrrole aldehydes in the presence of *p*-toluenesulfonic acid,[51] but tripyrrolic products could not be isolated in worthwhile yields. To overcome problems of this type, an alternative tripyrrene-*a,c*-biladiene route was developed[59] where the *tert*-butyl ester is cleaved prior to removal of the benzyl ester (Scheme 6). Treatment of **10** with TFA, followed by condensation with formylpyrroles **18** and HBr, gave the tripyrrene benzyl ester hydrobromide salts **23** as orange-red powders in 43−60% yield. Subsequent treatment with HBr−TFA for 6 h, followed by condensation with the complementary pyrrole aldehyde (i.e., the second aldehyde is different from the one used to prepare the tripyrrene), gave the *a,c*-biladiene dihydrobromide salts **24**. Cyclization with $CuCl_2$, followed by demetalation and reesterification, afforded the pentamethyl esters **25** and **26** in excellent yields. These are

(48) Clezy, P. S.; Hai, T. T.; Gupta, P. C. *Austr. J. Chem.* **1976**, *29*, 393.
(49) Arsenault, G. P.; Bullock, E.; MacDonald, S. F. *J. Am. Chem. Soc.* **1960**, *82*, 4384. Cavaleiro, J. A. S.; Rocha Gonsalves, A. M. d'A.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1771.
(50) Jackson, A. H.; Kenner, G. W.; McGillivray, G.; Smith, K. M. *J. Chem. Soc. C* **1968**, 294. Jackson, A. H.; Kenner, G. W. *Nature* **1967**, *215*, 1126.
(51) Baptista de Almeida, J. A. P.; Kenner, G. W.; Rimmer, R.; Smith, K. M. *Tetrahedron* **1976**, *32*, 1793.
(52) Kleinspehn, G. G. *J. Am. Chem. Soc.* **1955**, *77*, 1546.
(53) Plieninger, H.; Hess, P.; Ruppert, J. *Chem. Ber.* **1968**, *101*, 240.
(54) Jackson, A. H.; Pandey, R. K.; Rao, K. R. N.; Roberts, E. *Tetrahedron Lett.* **1985**, *26*, 793.
(55) Crook, P. J.; Jackson, A. H.; Kenner, G. W. *J. Chem. Soc. C* **1971**, 474. Battersby, A. R.; Hunt, E.; McDonald, E.; Paine, J. B., III; Saunders: J. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1008. Lash, T. D.; Bellettini, J. R.; Bastian, J. A.; Couch, K. B. *Synthesis* **1994**, 170.
(56) Drinan, M. A.; Lash, T. D. *J. Heterocycl. Chem.* **1994**, *31*, 255.
(57) Grigg, R.; Johnson, A. W.; Kenyon, R.; Math, V. B.; Richardson, K. *J. Chem. Soc. C* **1969**, 176.
(58) Lash, T. D.; Balasubramaniam, R. P. *Tetrahedron Lett.* **1990**, *31*, 7545. May, D. A., Jr.; Lash, T. D. *J. Org. Chem.* **1992**, *57*, 4820. Lash, T. D. *Tetrahedron* **1998**, *54*, 359.
(59) Smith, K. M.; Minnetian, O. M. *J. Chem. Soc., Perkin Trans. 1* **1986**, 277.

Scheme 6



the precursors to porphyrinogens **5abc** and **5bcd**, respectively.

Metabolism of Type-III Pentacarboxylate Porphyrinogens

Due to their inherent instability, porphyrinogens are generated immediately prior to use in a given biochemical experiment. Hence, porphyrin pentamethyl esters **20**, **21**, **25**, and **26** were treated with 25% hydrochloric acid for 16 h at room temperature and reduced under buffered conditions with 3% sodium−amalgam to generate the porphyrinogen pentacarboxylic acids. These products were incubated with CRH for different time periods and the products analyzed by TLC and normal-phase HPLC (analyses of the metabolic products were carried out on the esterified porphyrins derived from these materials). The kinetic profiles for these studies (Figure 1) consistently showed that **5dab** was by far the best substrate in these incubations in terms of the final amount of product produced, followed by **5abc**, while the remaining two pentacarboxylate porphyrinogens, **5acd** and **5bcd**, appeared to be equally poor substrates for UPD. The initial velocities for **5dab** and **5abc** were indistinguishable, although they were far greater for these substrates than for **5bcd** or **5acd**, but the maximum product formation was significantly greater for **5dab** (Table 1). Not only were these results highly reproducible, but similar data were also obtained for hemolysates derived from turkey and duck blood. It is interesting that **5abc** should be the second best substrate as this is the only pentacarboxylate porphyrinogen that could not be derived from **7d**, the first intermediate in the "clockwise" decarboxylation pathway. The striking differences in these kinetic profiles provide strong support for **5dab** being an intermediate in the preferred decarboxylation pathway leading to copro'gen-III.

In addition, the effect of varying substrate concentration on enzyme activity was assessed using **5dab** as substrate. Using a range from 2 to 19 μM, **5dab** was incubated for 10 min with CRH. The results (Table 2) clearly show that above 2 μM the substrate appears to be inhibitory for the enzyme. Others have also reported that high substrate concentrations were inhibitory for UPD.[9,14] Using uro'gen-III as substrate with preparations of UPD derived from *Rh. sphaeroides*, Jones and Jordan[14] reported inhibition at concentrations greater than 12 μM, and similar observations have been reported for chicken erythrocyte UPD by Kawanishi et al.[9] Taken together, these data are suggestive that during porphyria high levels of substrate can inhibit the activity of UPD and this may result in the processing of uro'gen-III via the nonpreferred (abnormal) hepta-, hexa-, and pentacarboxylate porphyrinogens.

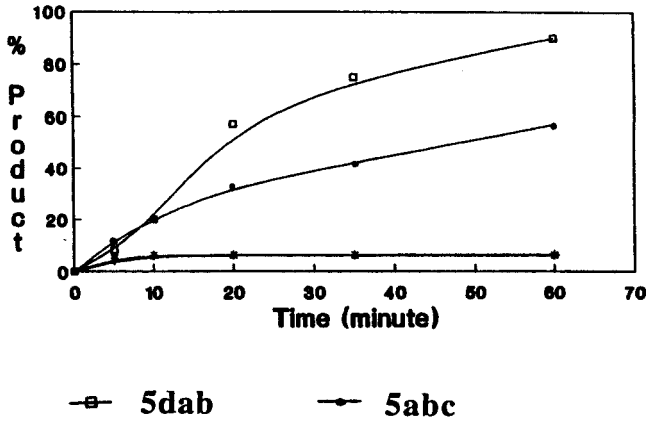


Figure 1. Time course study for incubations of pentacarboxylate porphyrinogens **5dab**, **5abc**, **5acd**, and **5bcd** with chicken red cell hemolysates showing percentage of product formation vs time. Product is defined as copro'gen-III (isolated as coproporphyrin-III) and its metabolites (harderoporphyrin and protoporphyrin-IX).

Table 1. Initial Velocities and Maximum Product Formation for the Four Synthetic Pentacarboxylate Porphyrinogen Substrates (mean ± SD[a])

substrates	initial velocity (% product/min)	% product formed[b]
5dab	2.0	90 ± 12
5abc	2.0	57 ± 11
5bcd	0.4	6 ± 3
5acd	0.4	6 ± 3

[a] For three replicate experiments. [b] After a 60 min incubation with CRH.

Table 2. Effect of 5dab Concentration on the % Product Formed by UPD[a]

concentration of **5dab** (μM)	% product[b]
2	66 ± 2.6
5	46 ± 3.5
8	26 ± 2.5
13	20 ± 3.5
19	12 ± 1.7

[a] 20 min incubations with CRH at 37 °C. [b] For three replicate experiments.

Conclusions

Although it was proposed over 20 years ago,[26] the "clockwise" decarboxylation hypothesis for the enzymic

conversion of uro'gen-III to copro'gen-III has remained controversial. We have synthesized the four possible pentacarboxylate intermediates and demonstrated that one isomer, **5dab**, is a significantly better substrate for uro'gen decarboxylase than the other three. These kinetic studies provide further support for the preferred decarboxylation pathway proposed by Jackson et al. Further investigations at the hexa- and heptacarboxylate levels should also give insights into the selectivity of this important metabolic process.

Experimental Section[61]

Enzyme incubations and analyses of metabolic products were carried out as described in the prequel to this paper,[1] except that HPLC analyses were carried out using 50% ethyl acetate/cyclohexane as the mobile phase.

Dibenzyl Malonate. A stirred mixture of malonic acid (104 g), benzyl alcohol (207 mL), *p*-toluenesulfonic acid (0.50 g), and toluene (300 mL) was heated under reflux in a round-bottom flask fitted with a Dean−Stark apparatus until 35.5 mL of water had been collected (theoretical 36 mL). The solvent was evaporated under reduced pressure, and subsequent vacuum distillation gave dibenzyl malonate (249.3 g; 87%) as a colorless oil: bp 180−185 °C/0.2 Torr (lit.[62] bp 188 °C/0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.22 (2H, s), 4.98 (4H, s), 7.2 (10H, s).

Dibenzyl Oximinomalonate (14).[29] Sodium hydroxide (20.0 g) was added with stirring to glacial acetic acid (160 mL). After the pellets were completely dissolved and the mixture remained hot, dibenzyl malonate (242.2 g) was added, and the dropwise addition of a solution of sodium nitrite (110.0 g) in water (150 mL) was immediately initiated. Once the addition was complete, the mixture was stirred overnight to ensure complete evolution of nitrogen dioxide. Water and ether were added so that all the precipitated material dissolved. The ethereal layer was separated, washed with water and 5% aqueous sodium bicarbonate solution, and dried over sodium sulfate and the solvent evaporated under reduced pressure to give oxime **14** (245.2 g; 92%) as a pale yellow oil that solidified on standing: ^{1}H NMR ($CDCl_3$) δ 5.20 (2H, s), 5.30 (2H, s), 7.3−7.6 (10H, m), 10.0 (1H, br).

Methyl 4,6-Dioxoheptanoate (12). A mixture of magnesium turnings (26.7 g), absolute ethanol (135 mL), and carbon tetrachloride (5 mL) was stirred until the evolution of hydrogen gas subsided (2 h). Dry benzene (200 mL) was added, and the mixture was heated under gentle reflux for 4 h or until hydrogen evolution was complete. The mixture was cooled to room temperature, and dry diethyl ether (500 mL) was added. *tert*-Butyl acetoacetate (170 mL) was then added dropwise at a rate sufficient to maintain gentle reflux. After the addition was complete, the solution was further refluxed for 30 min. The solution was cooled to room temperature, and 3-methoxycarbonylpropanoyl chloride[63] (207.9 g) was added dropwise to the stirred mixture. After the initial reaction had subsided, the solution was refluxed for 1 h and then stirred overnight at room temperature. The solution was cooled using an ice−salt bath and treated with 5 M sulfuric acid (200 mL) and water to dissolve any precipitates that had formed. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine and dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The residual oil was stirred with *p*-toluenesulfonic acid (1.20 g) at 140−150 °C until gas evolution was complete (approximately 2 h). Vacuum distillation gave the diketone (87.0 g; 50%) as a pale yellow oil: bp 75−80 °C/0.025 Torr.

Benzyl 4-[(Ethoxycarbonyl)methyl]-3-[2-(methoxycarbonyl)ethyl]-5-methylpyrrole-2-carboxylate (15). Ethyl bromoacetate (16.7 mL) was added dropwise to a stirred solution of dione **12** (35.00 g), potassium carbonate (23.40 g), and reagent-grade acetone (150 mL) while a gentle reflux was sustained. After the addition was complete, the solution was refluxed for 5 h and then filtered while hot. The solid was washed with acetone and the solvent evaporated under reduced pressure. The residue was dissolved in chloroform, washed thoroughly with water to remove all inorganic salts, dried over magnesium sulfate, and filtered and the solvent evaporated under reduced pressure. Vacuum distillation gave methyl 5-[(ethoxycarbonyl)methyl]-4,6-dioxoheptanoate (**13**) as a pale yellow oil (20.88 g; 40%): bp 125−138 °C/0.25 Torr.

A solution of dibenzyl oximinomalonate (40.47 g) in glacial acetic acid (80 mL) was added to a stirred solution of the foregoing dione (20.88 g) in glacial acetic acid (100 mL) at 70 °C, while a mixture of zinc dust (32.8 g) and sodium acetate (16.4 g) was slowly added and the reaction temperature was maintained near 90 °C. After the addition was complete, the mixture was heated on a boiling water bath for 1 h, cooled to 70 °C, and poured into 4 L of ice−water. The yellow oil was extracted into ether, washed with water, dried over magnesium sulfate, and filtered and the solvent evaporated under reduced pressure to give a yellow oil. The pyrrole was purified by chromatography on silica using a gradient elution of petroleum ether (60−90 °C), dichloromethane, and methanol (4:1:0 to 0:1:0.05). Fractions containing the product were combined, recrystallized from 95% methanol−water, and dried in vacuo to give the title pyrrole (12.3 g; 39%) as white crystals: mp 75−76 °C; IR (Nujol mull) ν 3316 (st, sh, NH), 1756, 1719, 1674 cm^{-1} (st, sh, 3 × CO); ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.3 Hz), 2.23 (3H, s), 2.55 (2H, t, J = 8.1 Hz), 3.02 (2H, t, J = 8.1 Hz), 3.42 (2H, s), 3.63 (3H, s), 4.12 (2H, q, J = 7.3 Hz), 5.29 (2H, s), 7.37 (5H, m), 9.00 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 11.70, 14.23, 20.70, 29.99, 34.85, 51.45, 60.80, 65.88, 96.17, 114.55, 116.71, 128.22, 128.36, 128.60, 130.86, 131.54, 136.22, 160.73, 171.75, 173.66. Anal. Calcd for $C_{21}H_{25}NO_6$: C, 65.10; H, 6.50; N, 3.61. Found: C, 65.00; H, 6.35; N, 3.46.

Benzyl 3-[2-(Methoxycarbonyl)ethyl]-4,5-dimethylpyrrole-2-carboxylate. Iodomethane (20.1 g) was added to a stirred mixture of **12** (27.19 g), potassium carbonate (15.2 g), and reagent-grade acetone (50 mL) in a round-bottom flask equipped with two condensers in tandem. The solution was stirred vigorously at 80 °C (oil bath) overnight. The hot mixture was filtered and washed with acetone and the solvent evaporated under reduced pressure. The residue was dissolved in chloroform, washed thoroughly with water to remove all the inorganic materials, dried over magnesium sulfate and filtered and the solvent evaporated under reduced pressure to give a yellow oil. Vacuum distillation gave methyl 5-methyl-4,6-dioxoheptanoate (21.70 g; 74%) as a pale yellow oil: bp 85−95 °C/0.025 Torr.

Dibenzyl oximinomalonate (30.1 g) in glacial acetic acid (50 mL) was added to the foregoing dione (18.4 g) in glacial acetic acid (60 mL) while a mixture of zinc dust (26.0 g) and sodium acetate (12.1 g) was added as described in the previous procedure. The organic layer was diluted with ether, washed with water, dried over magnesium sulfate, and filtered and the solvent evaporated under reduced pressure to give a yellow solid. Recrystallization from 95% methanol−water gave the title pyrrole as a white powder (10.1 g; 33%): mp 97−98 °C (lit.[64a] mp 93−94 °C; lit.[64b] mp 90−92 °C); IR (Nujol mull) ν 3304 (st, sh, NH), 1735, 1664 cm^{-1} (st, sh, 2 × CO); ^{1}H NMR ($CDCl_3$) δ 1.92 (3H, s), 2.15 (3H, s), 2.48 (2H, t, J = 8.2 Hz), 3.01 (2H, t, J = 8.2 Hz), 3.61 (3H, s), 5.27 (2H, s), 7.35 (5H, m), 8.83 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 8.30, 11.13, 20.60, 34.58, 51.13, 65.37, 115.81, 116.60, 127.80, 127.92, 128.25, 130.01, 130.22, 136.11, 160.66, 173.40.

Benzyl 5-(Acetoxymethyl)-4-[(ethoxycarbonyl)methyl]-3-[2-(methoxycarbonyl)ethyl]pyrrole-2-carboxylate (11). Lead tetraacetate (9.33 g) was added to a stirred solution of pyrrole **15** (8.00 g) in glacial acetic acid (200 mL) and acetic

(60) Smith, K. M.; Craig G. W. *J. Org. Chem.* **1983**, *48*, 4302.
(61) For general experimental procedures, see ref 1.
(62) Baker, B. R.; Schraub, R. E.; Querry, M. V.; Williams, J. H. *J. Org. Chem.* **1952**, *17*, 77.
(63) Cason, J. *J. Am. Chem. Soc.* **1942**, *64*, 1106.

anhydride (10 mL) and the solution stirred at room temperature for 2 days. The resulting mixture was poured into ice−water (1 L) and the precipitate filtered, dissolved in chloroform, and washed with water. The chloroform solution was filtered through Celite and dried over magnesium sulfate and the solvent evaporated under reduced pressure. Crystallization from 95% methanol−water gave the (acetoxymethyl)pyrrole (8.65 g; 94%) as a white powder: mp 108−108.5 °C; IR (Nujol mull) ν 3292 (st, br, NH), 1732, 1670 cm^{-1} (st, sh, 4 × CO); ^1H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 2.06 (3H, s), 2.54 (2H, t, J = 8.4 Hz), 3.01 (2H, t, J = 8.4 Hz), 3.52 (2H, s), 3.62 (3H, s), 4.12 (2H, q, J = 7.1 Hz), 5.05 (2H, s), 5.30 (2H, s), 7.36 (5H, s), 9.23 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 14.19, 20.54, 20.90, 29.68, 34.71, 34.90, 51.53, 56.92, 61.04, 66.21, 117.14, 119.09, 128.33, 128.42, 128.64, 128.92, 130.00, 135.91, 160.56, 171.48, 173.59. Anal. Calcd for $C_{23}H_{27}NO_8$: C, 62.01; H, 6.11; N, 3.14. Found: C, 61.71; H, 5.96; N, 3.19.

4-[2-(Methoxycarbonyl)ethyl]-3,5-dimethylpyrrole-2-carboxaldehyde (18a). Benzyl 4-[2-(methoxycarbonyl)ethyl]-3,5-dimethylpyrrole-2-carboxylate[65] (4.626 g), triethylamine (20 drops), and absolute ethanol (200 mL) were placed in a hydrogenation vessel. After the air had been displaced with a stream of nitrogen gas, 10% palladium charcoal (200 mg) was added. The mixture was shaken overnight at room temperature under an atmosphere of hydrogen at 40 psi. The catalyst was filtered off, and the solvent was evaporated under reduced pressure. The residue was taken up in dilute ammonia (5% w/v) and cooled to 0 °C and the solution neutralized with dilute HCl (5% w/v). The resulting precipitate was filtered, washed thoroughly with water to remove all traces of acid, and dried in vacuo to give 4-[2-(methoxycarbonyl)ethyl]-3,5-dimethylpyrrole-2-carboxylic acid (2.52 g; 76%) as a pale pink powder: mp 131−132 °C dec (lit.[66] mp 131 °C dec).

A solution of the foregoing pyrrolecarboxylic acid (1.40 g) was treated with trifluoroacetic acid (5 mL) for 10 min. Trimethyl orthoformate (3 mL) was added dropwise to the reaction mixture and the resulting mixture stirred for 10 min at 40 °C. The mixture was then cooled to room temperature, diluted with water (50 mL), and extracted with chloroform (3 × 50 mL). The combined organic layers were washed with dilute aqueous ammonia (5% v/v; 50 mL) and then again with water (50 mL). The organic layer was dried over magnesium sulfate and filtered and the solvent evaporated under reduced pressure. The residual oil was recrystallized from aqueous methanol to give the formylpyrrole (0.978 g; 75%) as green crystals: mp 125−127 °C (lit.[67a] mp 128−129 °C; lit.[67b] 123−125 °C); IR (Nujol mull) ν 3230 (st, sh, NH); 1732, 1634 cm^{-1} (st, sh, 2 × CO); ^1H NMR ($CDCl_3$) δ 2.26 (3H, s), 2.27 (3H, s), 2.45 (2H, t, J = 7.3 Hz), 2.72 (2H, t, J = 7.3 Hz), 3.67 (3H, s), 9.38 (1H, br s), 9.46 (1H, s); ^{13}C NMR ($CDCl_3$) δ 8.46, 11.20, 18.97, 34.30, 51.29, 120.67, 127.65, 132.22, 136.26, 173.05, 175.45.

3-[2-(Methoxycarbonyl)ethyl]-4,5-dimethylpyrrole-2-carboxaldehyde (18b). Benzyl 3-[2-(methoxycarbonyl)ethyl]-4,5-dimethylpyrrole-2-carboxylate (10.00 g) was hydrogenolyzed over 10% palladium−charcoal by the procedure described above. After the mixture was dried overnight in vacuo, 3-[2-(methoxycarbonyl)ethyl]-4,5-dimethylpyrrole-2-carboxylic acid (4.81 g; 77%) was obtained as a pale pink powder: ^1H NMR ($CDCl_3$) δ 1.93 (3H, s), 2.18 (3H, s), 2.56 (2H, t, J = 7.4 Hz), 3.03 (2H, t, J = 7.4 Hz), 3.66 (3H, s), 8.92 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 8.84, 11.75, 21.00, 34.90, 35.13, 51.76, 115.81, 117.71, 131.95, 132.45, 166.17, 174.21.

The foregoing carboxylic acid (1.40 g) was reacted with TFA (5 mL) and trimethyl orthoformate (3 mL) as described above. The crude product was purified by column chromatography on silica by elution with cyclohexane−ethyl acetate (1:1). Recrystallization from methanol gave the formylpyrrole (0.888 g; 68%) as brown needles: mp 91−93 °C (lit.[68] mp 93−94 °C; lit.[39] mp 92−92.5 °C); IR (Nujol mull) ν 3230 (st, sh, NH), 1732, 1634 cm^{-1} (st, sh, 2 × CO); ^1H NMR ($CDCl_3$) δ 1.96 (3H, s), 2.25 (3H, s), 2.56 (2H, t, J = 7.4 Hz), 3.02 (2H, t, J = 7.4 Hz), 3.68 (3H, s), 9.47 (1H, s), 10.0 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 9.39, 14.40, 20.09, 35.51, 51.60, 115.44, 123.53, 136.00, 136.43, 151.81, 173.34.

Benzyl 5′-(*tert*-Butoxycarbonyl)-3-[(ethoxycarbonyl)-methyl]-4,4′-bis[2-(methoxycarbonyl)ethyl]-3′-methyl-2,2′-dipyrrylmethane-5-carboxylate (10). Montmorillonite clay (K10; 2.70 g) was added to a stirred solution of (acetoxymethyl)pyrrole **11** (0.501 g) and *tert*-butyl 3-[2-(methoxycarbonyl)ethyl]-4-methylpyrrole-2-carboxylate (**16b**; 0.295 g) in dichloromethane (40 mL) and the mixture vigorously stirred at room temperature overnight. The clay catalyst was filtered off and the solvent evaporated under reduced pressure to give a pale pink solid. Recrystallization from methanol gave the title dipyrrylmethane (0.612 g; 83%) as a white solid: mp 150−151 °C (softens at 144 °C); ^1H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.2 Hz), 1.52 (9H, s), 1.99 (3H, s), 2.47−2.54 (4H, m), 2.99 (4H, t, J = 7.6 Hz), 3.45 (2H, s), 3.61 (3H, s), 3.66 (3H, s), 3.83 (2H, s), 4.15 (2H, q, J = 7.1 Hz), 5.25 (2H, s), 7.25−7.40 (5H, m), 8.74 (1H, br s), 9.23 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 8.80, 14.24, 20.61, 20.92, 23.01, 28.52, 29.72, 34.91, 35.08, 51.63, 61.43, 66.28, 80.73, 115.06, 116.98, 118.21, 119.69, 128.32, 128.54, 128.62, 128.87, 129.04, 130.68, 132.09, 136.23, 160.99, 161.05, 172.71, 173.90, 174.18. Anal. Calcd for $C_{35}H_{44}N_2O_{10}$: C, 64.40; H, 6.79; N, 4.29. Found: C, 64.40; H, 6.78; N, 4.25.

Dibenzyl 3-[(Ethoxycarbonyl)methyl]-3′,4-bis[2-(methoxycarbonyl)ethyl]-4′-methyl-2,2′-dipyrrylmethane-5,5-dicarboxylate (9). Prepared by the previous procedure from (acetoxymethyl)pyrrole **11** (2.50 g), benzyl 4-[2-(methoxycarbonyl)ethyl]-3-methylpyrrole-2-carboxylate (**16a**; 1.66 g) and Montmorillonite clay (K10; 13.50 g). Recrystallization from methanol gave the title dipyrrylmethane (3.21 g; 83%) as a white solid: mp 98−99 °C; ^1H NMR ($CDCl_3$) δ 1.21 (3H, t, J = 7.2 Hz), 2.28 (3H, s), 2.48 (2H, t), 2.57 (2H, t), 2.76 (2H, t), 3.01 (2H, t), 3.53 (2H, s), 3.51 (3H, s), 3.60 (3H, s), 3.92 (2H, s), 4.05 (2H, q, J = 7.2 Hz), 5.24 (2H, s), 7.25−7.41 (5H, m), 9.87 (1H, br s), 10.02 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 7.49, 10.82, 15.53, 17.36, 18.99, 26.40, 30.63, 31.62, 48.23, 48.70, 58.27, 62.06, 62.54, 111.57, 115.00, 115.19, 116.29, 123.84, 124.74, 125.04, 125.07, 125.15, 125.27, 125.44, 126.89, 127.97, 129.28, 133.17, 133.68, 157.61, 158.06, 170.56, 170.61, 171.77. Anal. Calcd for $C_{38}H_{42}N_2O_{10}$: C, 66.46; H, 6.16; N, 4.08. Found: C, 66.26; H, 6.18; N, 3.92.

5′-(*tert*-Butoxycarbonyl)-3-[(ethoxycarbonyl)methyl]-4,4′-bis[2-(methoxycarbonyl)ethyl]-3′-methyl-2,2′-dipyrrylmethane-5-carboxylic Acid (22). A mixture of dipyrrylmethane **10** (0.445 g), triethylamine (20 drops), and ethanol (100 mL) was shaken with 10% palladium−charcoal (50 mg) under a hydrogen atmosphere at 40 psi and room temperature for 16 h. The catalyst was filtered off and the solvent evaporated under reduced pressure. The residue was taken up in aqueous ammonia (5%) and cooled to 0 °C using an ice−salt bath. The solution was neutralized with glacial acetic acid, maintaining the temperature at 0 °C, and the resulting precipitate filtered off, washed thoroughly with water, and dried in vacuo overnight. The title dicarboxylic acid (428 mg; quantitative) was obtained as an off-white powder: mp 71−72 °C dec; ^1H NMR ($CDCl_3$) δ 1.25 (3H, t), 1.55 (9H, s), 2.01 (3H, s), 2.47 (2H, t), 2.61 (2H, t), 2.97 (2H, t), 3.06 (2H, t), 3.48 (2H, s), 3.65 (3H, s), 3.68 (3H, s), 3.91 (2H, s), 4.14 (2H, q), 10.79 (1H, br s), 11.33 (1H, br s). Anal. Calcd for $C_{28}H_{38}N_2O_{10}$: C, 59.77; H, 6.81; N, 4.98. Found: C, 59.69; H, 6.71; N, 4.76.

3-[(Ethoxycarbonyl)methyl]-3′,4-bis[2-(methoxycarbonyl)ethyl]-4′-methyl-2,2′-dipyrrylmethane-5,5′-dicarboxylic Acid (17). Dipyrrylmethane dibenzyl ester **9** (0.382 g),

(64) (a) Crook, P. J.; Jackson, A. H.; Kenner, G. W. *Liebigs Ann. Chem.* **1971**, *748*, 26. (b) Clezy, P. S.; Diakiw, V. *Aust. J. Chem.* **1975**, *28*, 2703.

(65) Johnson, A. W.; Markham, E.; Price, R.; Shaw, K. B. *J. Chem. Soc.* **1958**, 4254.

(66) Jackson, A. H.; Kenner, G. W.; Wass, J. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1475.

(67) (a) Davies, J. L. *J. Chem. Soc. C* **1968**, 1392. (b) Al-Hazimi, H. M. G.; Jackson, A. H.; Knight, D. W.; Lash, T. D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 265.

(68) Smith, K. M.; Pandey, R. K. *J. Heterocycl. Chem.* **1985**, *22*, 1041.

triethylamine (20 drops), acetone (200 mL), and 10% palladium-charcoal (50 mg) were placed in a hydrogenation vessel and hydrogenolyzed following the previous procedure. After being dried in vacuo overnight, the title pyrrole (0.257 g; 90%) was obtained as an off-white powder: mp 158.5−160 °C dec; ^{1}H NMR (DMSO-d_6): δ 1.14 (3H, t, J = 7.2 Hz), 2.12 (3H, s), 2.13 (2H, t), 2.43 (2H, t), 2.53 (2H, t), 2.79 (2H, t), 3.40 (2H, s), 3.55 (3H, s), 3.56 (3H, s), 3.80 (2H, s), 4.15 (2H, q, J = 7 Hz), 11.02 (1H, br s), 11.31 (1H, br s), 12.1 (2H, v br). Anal. Calcd for $C_{24}H_{30}N_2O_{10}$: C, 56.91, H, 5.97; N, 5.53. Found: C, 57.02; H, 6.10; N, 5.34.

Benzyl 3-[(Ethoxycarbonyl)methyl]-2,8,13-tris[2-(methoxycarbonyl)ethyl]-7,12,14-trimethyl-5,16-dihydrotripyrrin-1-carboxylate Hydrobromide (23a). Benzyl 5′-(*tert*-butoxycarbonyl)-3-[(ethoxycarbonyl)methyl]-4,4′-bis[2-(methoxycarbonyl)ethyl]-3′-methyl-2,2′-dipyrrylmethane-5-carboxylate (**10**; 0.511 g) was treated with trifluoroacetic acid (3.75 mL) with stirring for 5 min at ambient temperature. A solution of carboxaldehyde **18a** (0.187 g) in methanol (20 mL) was added all at once and the mixture stirred at room temperature for 90 min. A mixture of aqueous HBr (48%) and acetic acid (1:2.33 v/v, 0.1 mL) and ether (30 mL) was added, and the mixture was stirred for a further 1 h at room temperature and then placed in a freezer overnight. The precipitate was filtered, washed thoroughly with ether, and dried in vacuo overnight to give the title tripyrrene hydrobromide (0.403 g; 60.5%) as an orange-brown powder: mp 77−78 °C; UV−vis ($CHCl_3$) λ_{max} (log ϵ) 493 nm (4.93); ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t), 2.02 (3H, s), 2.35 (3H, s), 2.69 (3H, s), 2.51 (6H, m), 2.77 (2H, t), 2.9−3.0 (4H, m), 3.52 (2H, s), 3.59 (3H, s), 3.61 (3H, s), 3.67 (3H, s), 4.15 (2H, q), 4.35 (2H, s), 5.30 (2H, s), 7.31 (4H, m), 7.48 (2H, d), 10.82 (1H, br s), 13.13 (2H, br s). Anal. Calcd for $C_{41}H_{50}N_3O_{10}Br\cdot 0.6H_2O$: C, 59.06; H, 6.04, N, 5.04. Found: C, 58.64; H, 5.97; N, 5.03.

Benzyl 3-[(Ethoxycarbonyl)methyl]-2,8,12-tris[2-(methoxycarbonyl)ethyl]-7,13,14-trimethyl-5,16-dihydrotripyrrin-1-carboxylate Hydrobromide (23b). Dipyrrylmethane **10** (0.275 g) was treated with trifluoroacetic acid (2.0 mL) with stirring at ambient temperature for 5 min. A solution of pyrrole-2-carboxaldehyde **18b** (0.101 g) in methanol (5.0 mL) was added all at once, the dark greenish-orange solution stirred for an additional 90 min, and a mixture of aqueous HBr (48%) and acetic acid (1:2.3 v/v, 2 drops) added dropwise. Rapid but dropwise addition of ether (20 mL) and continuous stirring of the mixture did not result in precipitation of the tripyrrene product, so the mixture was left in the freezer overnight. The resulting precipitate was filtered, washed thoroughly with ether, and dried in vacuo overnight to give the desired tripyrrene hydrobromide (0.154 g; 43%) as bright orange-red crystals, mp 93.5−94.5 °C; UV−vis ($CHCl_3$) λ_{max} (log ϵ) 493 nm (4.92); ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t), 2.02 (6H, s), 2.64 (3H, s), 2.5−2.6 (6H, m), 2.95−3.08 (6H, m), 3.52 (2H, s), 3.60 (3H, s), 3.61 (3H, s), 3.63 (3H, s), 4.10 (2H, q), 4.35 (2H, s), 5.30 (2H, s), 7.2−7.3 (3H, m), 7.49 (3H, m), 10.84 (1H, br s), 13.11 (2H, br). Anal. Calcd for $C_{41}H_{50}N_3O_{10}Br\cdot 0.5H_2O$: C, 59.06; H, 6.04, N, 5.04. Found: C, 58.73; H, 5.90; N, 5.04.

12-[(Ethoxycarbonyl)methyl]-2,7,13,17-tetrakis[2-(methoxycarbonyl)ethyl]-1,3,8,18,19-pentamethyl-10,23-dihydrobilin dihydrobromide (24a). Benzyl 3-[(ethoxycarbonyl)-methyl]-2,8,13-tris[2-(methoxycarbonyl)ethyl]-7,12,14-trimethyl-5,16-dihydrotripyrrin-1-carboxylate hydrobromide (**23a**; 0.310 g) was treated with a mixture of 30% HBr−acetic acid (1.0 mL) and trifluoroacetic acid (5.0 mL) with stirring at ambient temperature for 6 h. A solution of aldehyde **18b** (80 mg) in methanol (18 mL) was added all at once and the mixture stirred for 40 min. Ether (50 mL) was added dropwise but rapidly and stirred for 30 min in an ice bath. The precipitate was filtered, washed thoroughly with ether, and dried in vacuo overnight to yield the a,c-biladiene (0.221 g; 61%) as red crystals: mp 165−166 °C; UV−vis ($CHCl_3$) λ_{max} (log ϵ) 455 (4.38), 524 nm (5.17); ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t), 1.94 (3H, s), 2.04 (3H, s), 2.36 (3H, s), 2.4−2.6 (8H, m), 2.71 (3H, s), 2.73 (3H, s), 2.78 (2H, t), 2.90 (2H, t), 2.98 (2H, t), 3.05 (2H, t), 3.62 (6H, s), 3.65 (3H, s), 3.68 (3H, s), 3.72 (4H, m), 5.20 (2H, s), 7.33 (1H, s), 7.56 (1H, s), 13.19 (1H, br s), 13.33 (1H, br s), 13.40 (1H, br s), 13.43 (1H, br s). Anal. Calcd for $C_{44}H_{58}N_4O_{10}Br_2\cdot H_2O$: C, 53.88, H, 6.16; N, 5.71. Found: C, 53.02; H, 5.84; N, 5.77.

12-[(Ethoxycarbonyl)methyl]-3,7,13,18-tetrakis[2-(methoxycarbonyl)ethyl]-1,2,8,17,19-pentamethyl-10,23-dihydrobilin Dihydrobromide (24b). Tripyrrene hydrobromide **23b** (300 mg) was treated with a mixture of 30% HBr−acetic acid (1.0 mL) and trifluoroacetic acid (5.0 mL) and stirred at room temperature for 6 h. A solution of pyrrole aldehyde **18a** (80 mg) in methanol (18 mL) was added all at once and the mixture stirred for an additional 40 min. Ether (50 mL) was added dropwise over a short period of time, and stirring was continued for 1 h. The ether was evaporated and the residue taken up in methanol−ether and allowed to crystallize in the freezer overnight. The resulting crystals were filtered and dried in vacuo overnight to give the *a*,*c*-biladiene (186 mg; 53%) as a red-brown powder: mp 166−167 °C; UV−vis ($CHCl_3$) λ_{max} (log ϵ) 455 (4.37), 524 nm (5.11); ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t), 1.93 (3H, s), 2.03 (3H, s), 2.38 (3H, s), 2.4−2.6 (8H, m), 2.69 (3H, s), 2.75 (3H, s), 2.78 (2H, t), 2.94 (4H, m), 3.02 (2H, t), 3.62 (6H, s), 3.64 (3H, s), 3.68 (3H, s), 3.65−3.75 (4H, m), 5.20 (2H, s), 7.38 (1H, s), 7.51 (1H, s), 13.18 (1H, br s), 13.30 (1H, br s), 13.40 (1H, br s), 13.45 (1H, br s). Anal. Calcd for $C_{44}H_{58}N_4O_{10}Br_2\cdot H_2O$: C, 53.88, H, 6.16; N, 5.71. Found: C, 53.39; H, 5.75; N, 5.54.

8-[(Ethoxycarbonyl)methyl]-2,7,12,18-tetrakis[2-(methoxycarbonyl)ethyl]-1,3,13,17,19-pentamethyl-10,23-dihydrobilin Dihydrobromide (19a). Dipyrrole **17** (250 mg) was dissolved in trifluoroacetic acid (1.0 mL) and stirred at room temperature for 10 min. A solution of pyrrole aldehyde **18a** (207 mg) in methanol (2.0 mL) was added, and the residual material was washed into the reaction flask with methanol (2.0 mL). HBr in acetic acid (30%; 0.8 mL) was immediately added, and the mixture was stirred for 30 min at room temperature. Anhydrous ether (17 mL) was added dropwise, after which the mixture was stirred at room temperature for a further 2 h. The resulting precipitate was filtered and rinsed with anhydrous ether to give the *a*,*c*-biladiene dihydrobromide (325 mg; 68%) as a brick red solid (325 mg; 68%): mp 156−157 °C dec; UV−vis ($CHCl_3$) λ_{max} (log ϵ) 451 (4.65), 522 (5.15); ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t), 2.05 (3H, s), 2.02 (2H, t), 2.09 (3H, s), 2.16 (3H, s), 2.45−2.55 (8H, m), 2.70−2.81 (6H, m), 2.86 (2H, t), 2.70 (3H, s), 2.73 (3H, s), 3.40 (3H, s), 3.61 (3H, s), 3.67 (6H, s), 3.74 (2H, q), 3.78 (2H, s), 5.24 (2H, s), 7.08 (1H, s), 7.41 (1H, s), 13.24 (1H, s) 13.37 (1H, s) 13.45 (1H, s), 13.54 (1H, s). Anal. Calcd for $C_{44}H_{58}N_4O_{10}Br_2\cdot H_2O$: C, 53.88, H, 6.16; N, 5.71. Found: C, 53.31; H, 6.02; N, 5.89.

8-[(Ethoxycarbonyl)methyl]-3,7,12,17-tetrakis[2-(methoxycarbonyl)ethyl]-1,2,13,18,19-pentamethyl-10,23-dihydrobilin Dihydrobromide (19b). This was prepared by the procedure detailed above from dipyrrole **17** (500 mg) and formylpyrrole **18b** (414 mg). The title *a*,*c*-biladiene dihydrobromide salt (347 mg; 73%) was obtained as a brick-red solid: mp > 260 °C (darkens at 170 °C); UV−vis ($CHCl_3$) λ_{max} (log ϵ) 453 (4.60), 524 (5.13); ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t), 2.04 (3H, s), 2.10 (3H, s), 2.29 (3H, s), 2.5−2.65 (8H, m), 2.70 (3H, s), 2.73 (3H, s), 2.86 (2H, t), 2.95−3.1 (6H, m), 3.40 (3H, s), 3.61 (3H, s), 3.64 (3H, s), 3.65 (3H, s), 3.72 (2H, q), 3.81 (2H, s), 5.24 (2H, s), 7.28 (1H, s), 7.59 (1H, s), 13.21 (1H, s) 13.35 (1H, s) 13.43 (1H, s), 13.53 (1H, s). Anal. Calcd for $C_{44}H_{58}N_4O_{10}Br_2\cdot H_2O$: C, 53.88, H, 6.16; N, 5.71. Found: C, 53.76; H, 5.70; N, 5.58.

2,8,12,17-Tetrakis[2-(methoxycarbonyl)ethyl]-7-[(methoxycarbonyl)methyl]-3,13,18-trimethylporphyrin. (25). *a*,*c*-Biladiene dihydrobromide **24a** (374 mg) was added to a stirred solution of copper(II) chloride (1.13 g) in *N*,*N*-dimethylformamide (135 mL), and the resulting mixture was stirred in the dark for 2 h. The mixture was diluted with dichloromethane (200 mL) and washed with water (2 × 250 mL). The aqueous layers were back-extracted with dichloromethane, and the combined organic layers were dried over sodium sulfate and filtered. The solvent was evaporated on a rotary evaporator under aspirator pressure and then the residual DMF removed using a vacuum pump. The solid residue was taken up in 15% v/v sulfuric acid−trifluoroacetic acid (46 mL)

and stirred in the dark at room temperature for 45 min. The reaction mixture was diluted with dichloromethane (185 mL) and washed with water (2 × 185 mL) and 5% aqueous sodium bicarbonate solution (185 mL). The organic layer was dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was then dissolved in 5% sulfuric acid−methanol (50 mL) and stirred in the dark overnight for reesterification. The reaction mixture was diluted with dichloromethane and washed with water and then with 5% aqueous sodium bicarbonate solution. The organic layer was dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was chromatographed on grade-III alumina column (2.2 cm × 16 cm), eluting with dichloromethane. The violet fraction was evaporated under reduced pressure and the residue recrystallized from chloroform−methanol to give the title porphyrin (189 mg; 63%) as purple crystals, mp 213.5−214.5 °C (lit.[29] mp 211−212 °C; lit.[48] mp 218−219 °C[48]); UV−vis ($CHCl_3$) λ_{max} (log ϵ) 402 (5.23), 500 (4.14), 535 (3.97), 567 (3.79), 623 (3.56); ^{1}H NMR ($CDCl_3$) δ −3.79 (2H, br s), 3.24−3.32 (6H, m), 3.33 (2H, t), 3.62 (3H, s), 3.66 (3H, s), 3.67 (3H, s), 3.68 (9H, s), 3.69 (3H, s), 3.80 (3H, s), 4.35−4.48 (8H, m), 5.11 (2H, s), 10.07 (2H, s), 10.16 (1H, s), 10.17 (1H, s); HRFABMS calcd for $C_{42}H_{48}N_4O_{10}$ + H 769.3449, found, 769.3454.

2,8,13,18-Tetrakis[2-(methoxycarbonyl)ethyl]-7-[(methoxycarbonyl)methyl]-3,12,17-trimethylporphyrin (26). This porphyrin was prepared from *a*,*c*-biladiene **24b** (230 mg) by the procedure described above. Recrystallization from chloroform−methanol gave the title porphyrin (77 mg; 42%) as violet crystals: mp 154.5−155.5 °C (lit.[48] mp 154−155 °C); UV−vis ($CHCl_3$) λ_{max} (log ϵ) 402 (5.26), 500 (4.14), 535 (3.98), 567 (3.81), 621 nm (3.59); ^{1}H NMR ($CDCl_3$) δ −3.80 (2H, br s), 3.25−3.36 (8H, m), 3.62 (3H, s), 3.65 (3H, s), 3.67 (9H, s), 3.68 (3H, s), 3.71 (3H, s), 3.77 (3H, s), 4.35−4.5 (8H, m), 5.09 (2H, s), 10.07 (1H, s), 10.08 (1H, s), 10.10 (1H, s), 10.16 (1H, s); HRFABMS: calcd for $C_{42}H_{48}N_4O_{10}$ + H 769.3449, found: 769.3461.

3,8,13,17-Tetrakis[2-(methoxycarbonyl)ethyl]-7-[(methoxycarbonyl)methyl]-2,12,18-trimethylporphyrin (20). The title porphyrin was prepared from *a*,*c*-biladiene **19a** (250 mg) by the procedure described above. Recrystallization from chloroform−methanol gave **20** as a purple solid (85 mg; 42%): mp 217−218 °C (lit.[29] mp 219−220 °C; lit.[48] mp 218−219 °C); UV−vis ($CHCl_3$) λ_{max} 402, 500, 536, 568, 622 nm; ^{1}H NMR ($CDCl_3$) δ −3.81 (2H, br s), 3.24 (8H, m), 3.55 (3H, s), 3.58 (3H, s), 3.59 (6H, s), 3.60 (3H, s), 3.62 (3H, s), 3.65 (3H, s), 3.71 (3H, s), 4.3−4.5 (8H, m), 5.05 (2H, s), 10.02 (1H, s), 10.03 (1H, s), 10.06 (1H, s), 10.10 (1H, s).

3,8,13,17-Tetrakis[2-(methoxycarbonyl)ethyl]-7-[(methoxycarbonyl)methyl]-2,12,18-trimethylporphyrin (21). *a*,*c*-Biladiene dihydrobromide **19b** (400 mg) was cyclized by the procedure detailed above. Recrystallization from chloroform−methanol gave the title porphyrin as a purple solid (196 mg; 61%): mp 209.5−211 °C (lit.[29] mp 205−206 °C; lit.[48] mp 210−211 °C; lit.[51] mp 214−215.5 °C); UV−vis ($CHCl_3$) λ_{max} 402, 500, 536, 568, 622 nm; ^{1}H NMR ($CDCl_3$) δ −3.81 (2H, br s), 3.25 (8H, m), 3.55 (3H, s), 3.57 (3H, s), 3.60 (12H, s), 3.62 (3H, s), 3.70 (3H, s), 4.3−4.5 (8H, m), 5.06 (2H, s), 9.99 (1H, s), 10.02 (1H, s), 10.09 (1H, s), 10.11 (1H, s); HRMS calcd for $C_{42}H_{48}N_4O_{10}$ 768.3370, found 768.3367.

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Supporting Information Available: Copies of proton NMR and selected carbon-13 NMR spectra for compounds **9**−**11** and **15**−**26** are provided (28 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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