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Normal and Abnormal Heme Biosynthesis. 6. Synthesis and Metabolism of a Series of Monovinylporphyrinogens Related to Harderoporphyrinogen. Further Insights into the Oxidative Decarboxylation of Porphyrinogen Substrates by Coproporphyrinogen Oxidase†

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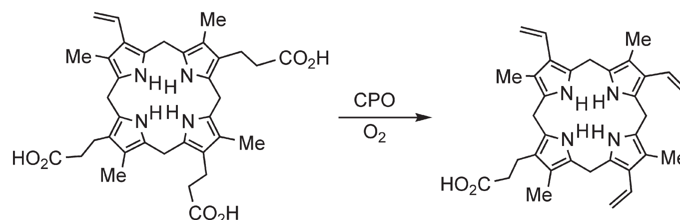

A series of vinylporphyrinogens were prepared to probe the enzyme coproporphyrinogen oxidase (CPO). Six (2-chloroethyl)porphyrins were synthesized from a common dipyrrylmethane via *a*,*c*-biladiene intermediates in excellent yields. Subsequent dehydrohalogenation with DBU in refluxing DMF then gave the required vinylporphyrin methyl esters, including harderoporphyrin-I, harderoporphyrin-III, and isoharderoporphyrin. The corresponding porphyrinogen carboxylic acids were incubated with chicken red cell hemolysates, which contain the enzyme CPO, and the products analyzed. The 17-ethyl analogue of harderoporphyrinogen-III, but not its 13-ethyl isomer, was shown to be an excellent substrate for CPO in accord with a proposed model for the active site of this enzyme. In addition, harderoporphyrinogen-VII, the monovinyl intermediate in the metabolism of coproporphyrinogen-IV, was shown to be an equally good substrate for this enzyme. However, isoharderoporphyrinogen, which lacks the correct ordering of peripheral substituents, was also a substrate for CPO. Furthermore, a nonnatural type I isomer of harderoporphyrinogen was shown to be acted on by CPO, but in this case further metabolism was noted and this afforded an unprecedented trivinyl porphyrinogen product. The corresponding porphyrin methyl ester was isolated and characterized by FAB MS and proton NMR spectroscopy. The results from these studies allow the binding requirements of CPO to be further assessed and provide a series of substrates to investigate this poorly understood enzyme.

Introduction

Uroporphyrinogen-III (uro'gen-III, Scheme 1) is the first macrocyclic intermediate in the biosynthesis of the hemes, chlorophylls, and corrins (e.g., vitamin B_{12}),[1-4] although the pathway to the corrins and related tetrapyrroles such as siroheme diverges at this point.[5] In vertebrates, uroporphyrinogen-III

† Part 5: Cooper, C. L.; Stob, C. M.; Jones, M. A.; Lash, T. D. *Bioorg. Med. Chem.* **2005**, *13*, 6244−6251.

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

(2) (a) Dailey, H. A. *Biochem. Soc. Trans.* **2002**, *30*, 590–599. (b) Ajioka, R. S.; Phillips, J. D.; Kushner, J. P. *Biochim. Biophys. Acta* **2006**, *1763*, 723–736.

(3) Jones, M. A.; Lash, T. D. *Curr. Top. Biotechnol.* **2004**, *1*, 15–27.

(4) (a) Akhtar, M. The Modification of Acetate and Propionate Side Chains during the Biosynthesis of Haem and Chlorophylls: Mechanistic and Stereochemical Studies. In *The Biosynthesis of Tetrapyrrolic Pigments*; Ciba Foundation Symposium 180; Wiley: Chichester, 1994; pp 130−155. (b) Akhtar, M. Coproporphyrinogen III and Protoporphyrinogen IX Oxidase. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Elsevier: San Diego, 2003; Vol. 12, pp 75−92. (c) Lewis, C. A., Jr.; Wolfenden, R. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17328–17333.

(5) (a) Scott, A. I. *Acc. Chem. Res.* **1990**, *23*, 308–317. (b) Battersby, A. R. *Acc. Chem. Res.* **1993**, *26*, 15–21.

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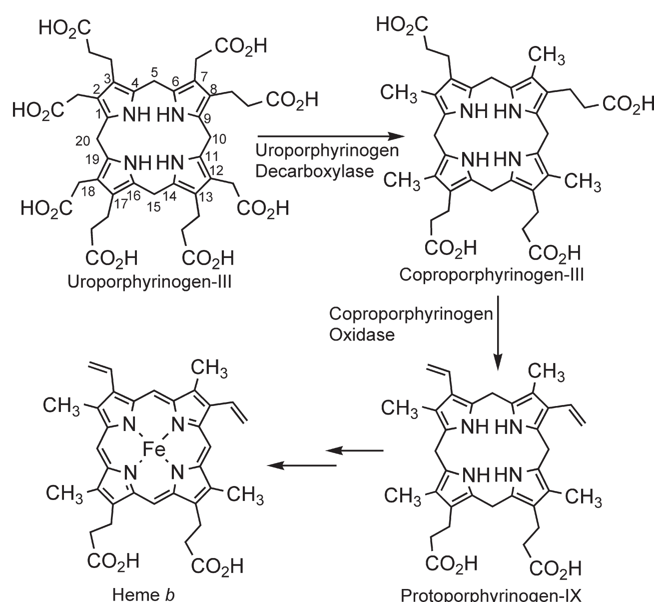
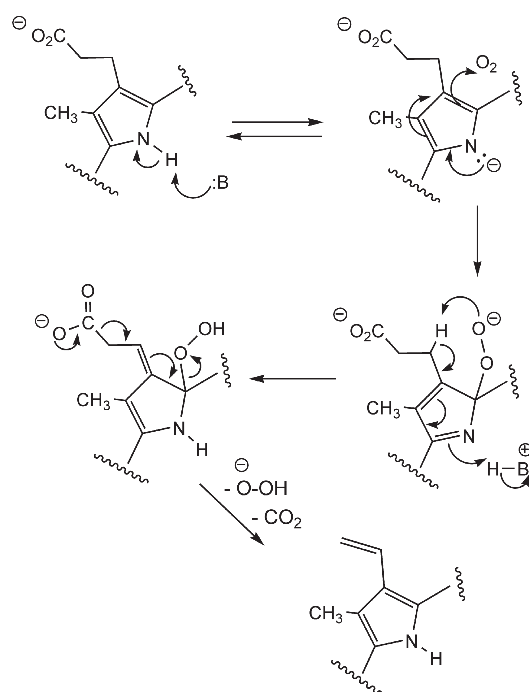
SCHEME 1



SCHEME 2



undergoes a sequential decarboxylation process mediated by the cytoplasmic enzyme uroporphyrinogen decarboxylase (URO-D, E.C. 4.1.1.37) to afford the tetracarboxylate intermediate coproporphyrinogen-III (copro'gen-III).[6−9] Following transfer to the mitochondria, oxidative decarboxylation of two of the propionate side chains by coproporphyrinogen oxidase (CPO, E.C. 1.3.3.3) gives protoporphyrinogen-IX (proto'gen-IX).[4b,10] Dehydrogenation mediated by protoporphyrinogen oxidase affords the aromatic tetrapyrrole protoporphyrin-IX, and insertion of iron(II) by ferrochelatase then yields heme *b*.[1−4] Protoporphyrin-IX is also the precursor to many other hemes and chlorophylls.[1,11] Most of the key steps in the heme biosynthesis have been known for over 50 years,[1] but a detailed understanding of the individual enzyme-mediated processes remains somewhat incomplete. URO-D mediates the decarboxylation of four acetate side chains by a well-understood mechanistic process,[4a,c] but the specificity of the decarboxylation pathway[7−9] and the potential interplay of the two protein subunits[12] still requires further study. CPO is a more enigmatic enzyme that converts two propionic acid side chains into vinyl groups by a poorly understood oxidative decarboxylation process.[3,4,10] In aerobic organisms, CPO requires molecular oxygen for activity, but it is not a metalloprotein and does not utilize any cofactors.[13] It had been demonstrated that the propionate side chains are either initially hydroxylated so that dehydration with concomitant decarboxylation can give rise to the vinyl moieties[4,14] or that a hydride acceptor was involved.[4,15] However, these speculations are not consistent with the available data for aerobic CPO.[13] A structurally unrelated version of CPO is found in anaerobic organisms that incorporates a 4Fe−4S cluster and appears to generate protoporphyrinogen-IX by a radical pathway,[16] but this type of mechanism is not plausible for the oxygen-dependent form of CPO.[17] One of us recently proposed a new mechanism that involves the base-catalyzed generation of a peroxide anion intermediate from molecular oxygen, followed by intramolecular deprotonation of the propionate unit and loss of H_2O_2 and CO_2 (Scheme 2).[17] The formation of hydrogen peroxide and carbon dioxide as byproduct from the metabolism of copro'gen-III has been demonstrated,[18,19] and the new mechanism has received further support from independent DFT calculations.[20] In addition, this mechanism resembles the one proposed for the conversion of urate to 5-hydroxyisourate by urate oxidase using molecular oxygen, again without the

(6) (a) 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*, 191–206. (b) Shoolingin-Jordan, P. M. The Biosynthesis of Coproporphyrinogen III. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Elsevier: San Diego, 2003; Vol. 12 pp 33−74.

(7) Lash, T. D. *Biochem. J.* **1991**, *278*, 901–903.

(8) Luo, J.; Lim, C. K. *Biochem. J.* **1993**, *289*, 529–532.

(9) Lash, T. D.; Mani, U. N.; Lyons, E. A.; Thientanavich, P.; Jones, M. A. *J. Org. Chem.* **1999**, *64*, 478–487.

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

(11) Interestingly, an alternative pathway for the biosynthesis of C-type hemes has been discovered in the primative anaerobic bacterium *Desulfovibrio vulgaris*. See: (a) Akutsu, H.; Park, J.-S.; Sano, S. *J. Am. Chem. Soc.* **1993**, *115*, 12185–12186. (b) Ishida, T.; Yu, L.; Akutsu, H.; Ozawa, K.; Kawanishi, S.; Seto, A.; Inubushi, T.; Sano, S. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4853–4858. (c) Lobo, S. A. L.; Brindley, A.; Warren, M. J.; Saralva, L. M. *Biochem. J.* **2009**, *420*, 317–325.

(12) Phillips, J. D.; Warby, C. A.; Whitby, F. G.; Kushner, J. P.; Hill, C. P. *J. Mol. Biol.* **2009**, *389*, 306–314.

(13) Medlock, A. E.; Dailey, H. A. *J. Biol. Chem.* **1996**, *271*, 32507–32510.

(14) (a) Sano, S. *J. Biol. Chem.* **1966**, *241*, 5276–5283. (b) Yoshinaga, T.; Sano, S. *J. Biol. Chem.* **1980**, *255*, 4727–4731.

(15) Seehra, J. S.; Jordan, P. M.; Akhtar, M. *Biochem. J.* **1983**, *209*, 709–718.

(16) (a) Layer, G.; Grage, K.; Teschner, T.; Schunemann; Breckau, D.; Masoumi, A.; Jahn, M.; Heathcote, P.; Trautwein, A. X.; Jahn, D. *J. Biol. Chem.* **2005**, *280*, 29038–29046. (b) Layer, G.; Pierik, A. J.; Trost, M.; Rigby, S. E.; Leech, H. K.; Grage, K.; Breckau, D.; Astner, I.; Jansch, L.; Heathcote, P.; Warren, M. J.; Heinz, D. W.; Jahn, D. *J. Biol. Chem.* **2006**, *281*, 15727–15734.

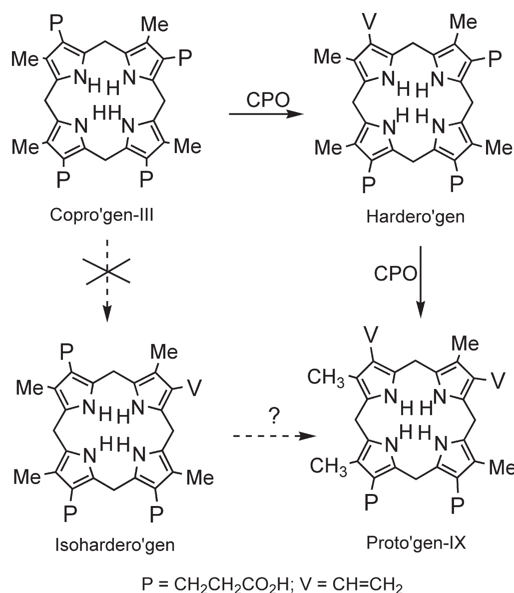
(17) Lash, T. D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4506–4509.

(18) Breckau, D.; Mahlitz, E.; Sauerwald, A.; Layer, G.; Jahn, D. *J. Biol. Chem.* **2003**, *278*, 46625–46631.

(19) Elder, G. H.; Evans, J. O. *Biochem. J.* **1978**, *169*, 205–214.

(20) Silva, P. J.; Ramos, M. J. *Bioorg. Med. Chem.* **2008**, *16*, 2726–2733.

SCHEME 3



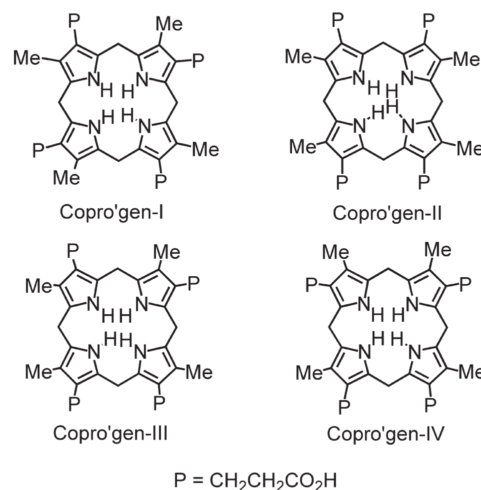


FIGURE 1. Type isomers of coproporphyrinogen.

involvement of transition metal ions or cofactors.[21] A better understanding of CPO is needed, not only due to its central role in heme and chlorophyll biosynthesis but also because defects in this enzyme can lead to a disease state known as hereditary coproporphyria.[22] This disease may result in the overproduction and accumulation of porphyrins and can lead to skin photosensitivity and neurological disorders.[22]

It is known that CPO carries out the two oxidative decarboxylations sequentially to give a monovinylporphyrinogen intermediate (Scheme 3).[1-4] This process is regiospecific, generating harderoporphyrinogen (hardero'gen) as an intermediate by first converting the A ring side chain to a vinyl unit.[1-4] The corresponding porphyrin was first isolated and characterized from the harderian glands of rats[23] and the structure was confirmed by total synthesis.[24] Harderoporphyrin and metabolites have also been observed in extracts from urine and feces,[25-27] and although isomeric porphyrins have been detected, these appear to arise from isomerization of the porphyrinogens prior to oxidation.[27] In enzyme incubation studies using copro'gen-III, the formation of hardero'gen (or the related porphyrin) has be demonstrated by HPLC[24,28] and MS studies,[29] but the regioisomer isohardero'gen (Scheme 3) has not been detected. Nevertheless, isohardero'gen has been reported to be a substrate for CPO.[14,30] In order to assess the selectivity and binding requirements for CPO, isomeric copro'gens were investigated. There are four "type isomers" of copro'gen (Figure 1), including the natural "type III" isomer.[31] The type I and II isomers are not metabolized by CPO,[32] but copro'gen-IV is a good substrate for this enzyme.[32-37] Copro'gen-IV initially forms a type IV isomer of hardero'gen, and this is further converted into proto'gen-XIII (Scheme 4).[33-37] URO-D metabolizes all four uro'gen isomers, as well as all of the possible hepta-, hexa-, and pentacarboxylate porphyrinogens for the type I and III series[6-9,38] and for this reason is considered to be a relatively promiscuous enzyme compared to CPO.[4] However, CPO has been shown to metabolize a series of substrate analogues **1a**–**d** (Scheme 5) where the 13 and 17-propionate residues have been replaced by methyl, ethyl, propyl, or butyl groupings.[10,39-41] An early report suggested that the diethylporphyrinogen **1a** (meso'gen-VI) was converted to a divinylic product,[39] but extensive studies using **1a**–**d** with crude avian CPO preparations or purified human recombinant CPO have afforded only the monovinyl products **2a**–**d**.[10,42]

(21) (a) Kahn, K.; Serfozo, P.; Tipton, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 5435–5442. (b) Kahn, K.; Tipton, P. A. *Biochemistry* **1998**, *37*, 11651–11659.

(22) (a) Moore, M. R. *Int. J. Biochem.* **1993**, *25*, 1353–1368. (b) McDonagh, A. F.; Bissell, D. M. *Semin. Liver Dis.* **1998**, *18*, 3. (c) Martesek, P. *Semin. Liver Dis.* **1998**, *18*, 25–32. (d) McCarroll, N. A. *Anal. Chem.* **1995**, *67*, 425–428.

(23) Kennedy, G. Y.; Jackson, A. H.; Kenner, G. W.; Suckling, C. J. *FEBS Lett.* **1970**, *6*, 9–12.

(24) (a) Cavaleiro, J. A. S.; Kenner, G. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1188–1194. (b) Kenner, G. W.; Quirke, J. M. E.; Smith, K. M. *Tetrahedron* **1976**, *32*, 2753–2756.

(25) (a) Smith, S. G.; Belcher, R. V.; Mahler, R. F.; Yudkin, J. *Clin. Chim. Acta* **1969**, *23*, 241–243. (b) Belcher, R. V.; Smith, S. G.; Mahler, R. F. *Enzyme* **1974**, *17*, 53–60.

(26) Jackson, A. H.; Rao, K. R. N.; Smith, S. G.; Lash, T. D. *Biochem. J.* **1985**, *227*, 327–331.

(27) Danton, M.; Lim, C. K. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2309–2316.

(28) Evans, N.; Jackson, A. H.; Matlin, S. A.; Towill, R. *J. Chromatogr.* **1976**, *125*, 345–355.

(29) Jackson, A. H.; Jones, D. M.; Philip, G.; Lash, T. D.; Batlle, A. M.; del, C.; Smith, S. G. *Int. J. Biochem.* **1980**, *12*, 681–688.

(30) Games, D. E.; Jackson, A. H.; Jackson, J. R.; Belcher, R. V.; Smith, S. G. *J. Chem. Soc., Chem. Commun.* **1976**, 187–188.

(31) Smith, K. M. In *Porphyrins and Metalloporphyrins*; Elsevier: Amsterdam, 1975; pp 3–28.

(32) Porra, P. J.; Falk, J. E. *Biochem. J.* **1964**, *90*, 69–75.

(33) (a) Buldain, G.; Hurst, J.; Frydman, R. B.; Frydman, B. *J. Org. Chem.* **1977**, *42*, 2953–2956. (b) Buldain, G.; Diaz, L.; Frydman, B. *J. Org. Chem.* **1977**, *42*, 2957–2960.

(34) Al-Hazimi, H. M. G.; Jackson, A. H.; Ryder, D. J.; Elder, G. H.; Smith, S. G. *J. Chem. Soc., Chem. Commun.* **1976**, 188–189.

(35) Al-Hazimi, H. M. G.; Jackson, A. H.; Knight, D. W.; Lash, T. D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 265–276.

(36) Battersby, A. R.; Hamilton, A. D.; McDonald, E.; Mombelli, L.; Wong, O.-H. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1283–1289.

(37) Cooper, C. L.; Lash, T. D.; Jones, M. A. *Med. Sci. Monit.* **2005**, *11*, BR420–425.

(38) Jackson, A. H.; Supphayen, D. M. *J. Chem. Soc., Perkin Trans. 1* **1987**, 277–286.

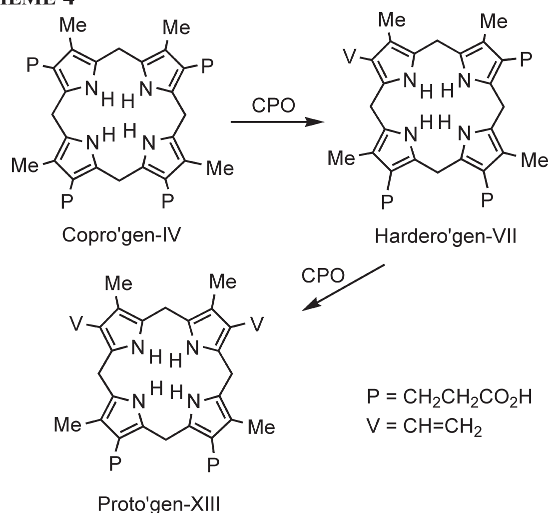
(39) Jackson, A. H.; Jones, D. M.; Smith, S. G.; Elder, G. H. *J. Chem. Soc., Chem. Commun.* **1976**, 322–323.

(40) Lash, T. D.; Drinan, M. A.; Zhen, C.; Mani, U. N.; Jones, M. A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1607–1612.

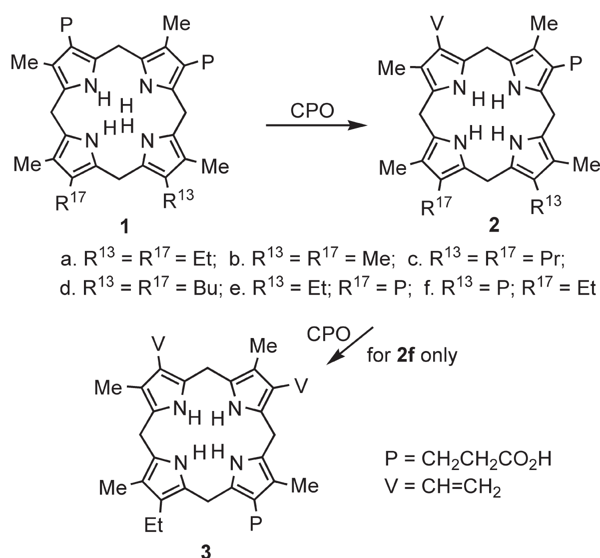
(41) Lash, T. D.; Hall, T.; Mani, U. N.; Jones, M. A. *J. Org. Chem.* **2001**, *66*, 3753–3759.

(42) Jones, M. A.; He, J.; Lash, T. D. *J. Biochem.* **2002**, *131*, 201–205.

SCHEME 4



SCHEME 5



In addition, when only the 13-propionate group is replaced by an ethyl (13-Et porphyrinogen **1e**), avian CPO still only processes the substrate once to give **2e**.[41,42] However, while the isomeric 17-Et porphyrinogen **1f** is an equally good substrate for CPO, it is further converted into the divinylporphyrinogen product **3**.[41,42] These and related results[32−37,43−46] were used to develop a model for the substrate binding sites in CPO (Figure 2).[10,17] In this model, three regions were designated. Region Y corresponds to the catalytic site where the propionate group is converted into the vinyl moiety, region X designates a site that requires the presence of a second propionate side chain for substrate binding, and position Z represents a region that can accommodate the presence of small nonpolar groups such

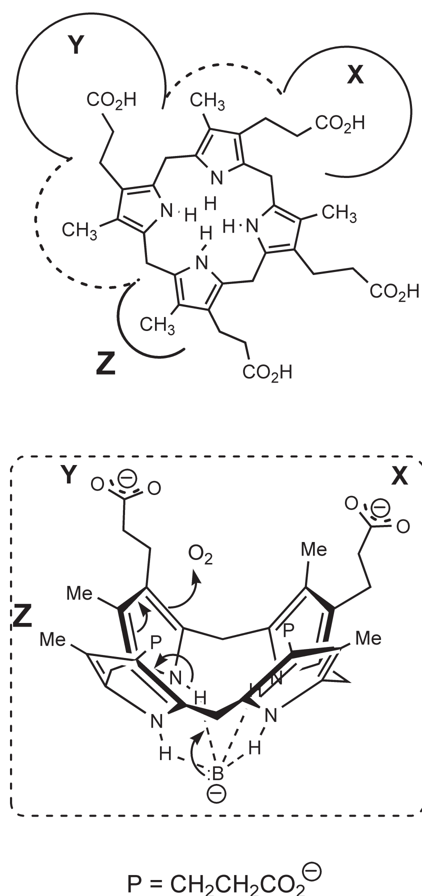


FIGURE 2. Active-site models for substrate binding by coproporphyrinogen oxidase. The top version shows a 2D representation where Y is the catalytic site, X recognizes and binds a second propionate residue, and Z can accommodate only small nonpolar groups like Me, Et, V, or H. The lower version envisages that the porphyrinogen binds as a bowl-shaped conformation and illustrates the proposed reaction with molecular oxygen.

as H, Me, vinyl (V), or Et but not propionate or acetate groupings. In order for a porphyrinogen to fit these binding requirements, it must possess the sequence of peripheral substituents R Me-P Me-P, where R is one of the small nonpolar groups listed above and P = $CH_2CH_2CO_2H$.[10]

In 2004, a crystal structure for the oxygen-dependent form of CPO from *Saccharomyces cerevisiae* was published,[47] and a similar structure was subsequentially reported for human recombinant CPO.[48] These were obtained as homodimers[49] that generate a nonpolar cleft that has been tentatively assigned as the active site.[47] Site-directed mutagenesis studies were used to demonstrate that the invariant amino acids aspartate 400, arginine 262, and arginine 401 were essential for significant catalytic activity,[50] and it was speculated that

(43) Jackson, A. H.; Lash, T. D.; Ryder, D. J.; Smith, S. G. *Int. J. Biochem.* **1980**, *12*, 775–780.
(44) Jackson, A. H.; Lash, T. D.; Ryder, D. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 287–298.
(45) Cooper, C. L.; Stob, C. M.; Jones, M. A.; Lash, T. D. *Bioorg. Med. Chem.* **2005**, *13*, 6244–6251.
(46) Lash, T. D.; Kaprak, T. A.; Shen, L.; Jones, M. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 181–186.
(47) Phillips, J. D.; Whitby, F. G.; Warby, C. A.; Labbe, P.; Yang, P.; Pflugrath, J. W.; Ferrara, J. D.; Robinson, H. A.; Kushner, J. P.; Hill, C. P. *J. Biol. Chem.* **2004**, *279*, 38960–38968.
(48) Lee, D. S.; Flacksova, E.; Bodnarova, M.; Demeler, B.; Martasek, P.; Raman, C. S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 14232–14237.
(49) Martasek, P.; Camadro, J.-M.; Raman, C. S.; Lecompte, M. C.; Le Caer, J. P.; Demeler, B.; Grandchamp, B.; Labbe, P. *Cell Mol. Biol.* **1997**, *43*, 47–58.
(50) Stephenson, J. R.; Stacey, J. A.; Morgenthaler, J. B.; Friesen, J. A.; Lash, T. D.; Jones, M. A. *Protein Sci.* **2007**, *16*, 401–410.

these residues could be involved in substrate binding and deprotonation of a pyrrolic NH to initiate reaction with molecular oxygen.[50,51] Porphyrinogens appear to bind with URO-D in a bowl-shaped conformation,[52] and it has been speculated that this is also the case for CPO.[17,47,50,53] This hypothesis has been used to modify the proposed model for substrate binding (Figure 2)[17] and parallels observations for anion binding by synthetic calix[4]pyrroles.[54] Despite all of these recent advances, our understanding of the precise mode for substrate binding and oxidative decarboxylation by CPO remains incomplete. In order to further develop these studies, a series of harderoporphyrinogens were required in our investigations. Relatively few studies have been carried out using vinylporphyrinogens, due in part to their decreased stability and the more stringent requirements for synthesis.[24,33−36] In this paper, we report new syntheses of the related harderoporphyrins and their activity as substrates for avian CPO.[55]

Results and Discussion

Hardero'gen has one pyrrole unit substituted with Me-V and three with Me-P (Figure 3). These units can be arranged to give a total of 8 type isomers which are numbered in accord with the principles originally developed by Hans Fischer.[31] Hardero'gens I and II are structurally related to copro'gens I and II, respectively, although neither one of these porphyrinogens can be formed by the action of CPO.[32] There are four type III hardero'gens (III−VI) and two type IV hardero'gens (VII and VIII). By this nomenclature, the natural isomer is hardero'gen-III, isohardero'gen is hardero'gen-IV, and the intermediate derived from copro'gen-IV is hardero'gen-VII. These designations will be used throughout the discussion, although the name isohardero'gen will be used instead of hardero'gen-IV to avoid confusion with copro'gen-IV. Isomers III and VII are known to be substrates for CPO,[32−36] and isohardero'gen is also reported to be a significant, albeit poorer, substrate.[30] Although the V- and VI-type isomers have not been synthesized, the related dihydrohardero'gens **1e** and **1f** (Scheme 5) are known substrates, and this strongly implies that hardero'gens V and VI would also be metabolized by CPO. Hardero'gens I and VIII also have the correct sequence of substituents to be substrates for CPO, and only isomer II is likely to be a nonsubstrate for this enzyme.[4] Therefore, it is a little surprising to find that 7 of the 8 type isomers of hardero'gen are likely to be substrates for this supposedly selective enzyme. To further our investigations, samples of hardero'gen-III and isohardero'gen were required. In addition, hardero'gen-I was selected as an intriguing potential substrate for CPO. Copro'gen-I is not a substrate for CPO, but if it were converted hardero'gen-I would be formed. Ironically, our model suggests that hardero'gen-I should be a good substrate for CPO and may

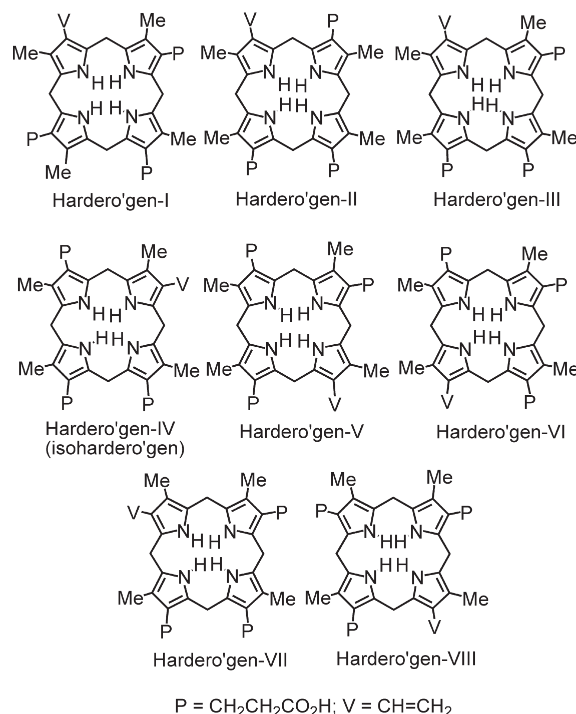
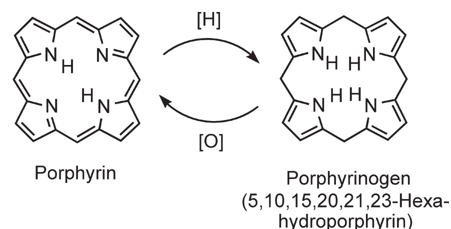


FIGURE 3. Eight type isomers of harderoporphyrinogen.

SCHEME 6



be converted into a trivinyl product (see below). The synthesis of hardero'gen-VII was also conducted to further investigate metabolism in the type IV series, and the monovinylic porphyrinogens **2e** and **2f** that are formed from the 13- or 17-ethyl porphyrinogens **1e** and **1f** were also needed for study.

The substrates for CPO are hexahydroporphyrins or porphyrinogens (Scheme 6), but these types of reduced tetrapyrroles are highly unstable and prone to oxidations and acid-catalyzed rearrangements.[56] For this reason, the corresponding porphyrins were targeted for synthesis. Porphyrins can easily be reduced to porphyrinogens (e.g., with sodium amalgam[10,57]), and this conversion could be carried out immediately prior to the biochemical studies. The porphyrins were also conveniently prepared as methyl esters and only converted into the corresponding carboxylic acids immediately prior to reduction.[10] Harderoporphyrin-III, isoharderoporphyrin, and harderoporphyrin-VII have been synthesized previously,[24,33a,35,36] but harderoporphyrin-I

(51) For related studies, see: Gitter, S. J.; Cooper, C. L.; Friesen, J. A.; Lash, T. D.; Jones, M. A. *Med. Sci. Monit.* **2007**, *13*, BR1–10.

(52) Phillips, J. D.; Whitby, F. G.; Kushner, J. P.; Hill, C. P. *EMBO J.* **2003**, *22*, 6225–6233.

(53) He, J.; Kaprak, T. A.; Jones, M. A.; Lash, T. D. *J. Porphyrins Phthalocyanines* **2005**, *9*, 170–185.

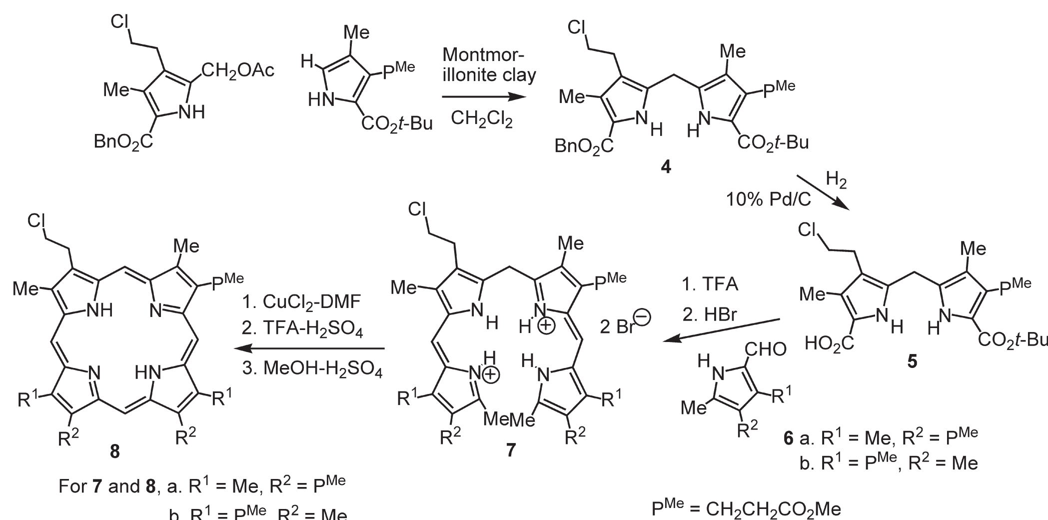
(54) (a) Gale, P. A.; Sessler, J. L.; Kral, V. *Chem. Commun.* **1998**, 1–8. (b) Blas, J. R.; Lopez-Bez, J. M.; Marquez, M.; Sessler, J. L.; Luque, F. J.; Orozco, M. *Chem.—Eur. J.* **2007**, *13*, 1108–1116.

(55) Preliminary communication: Lash, T. D.; Keck, A.-S.; I., M.; Mani, U. N.; Jones, M. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1079–1082.

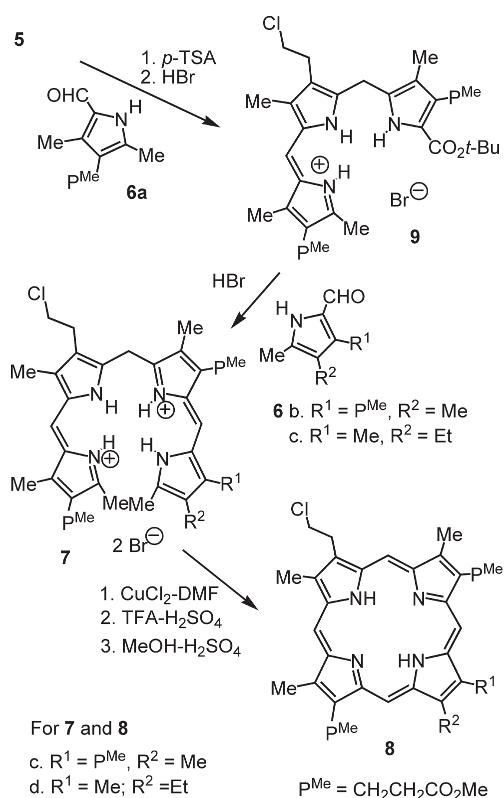
(56) (a) Mauzerall, D. *J. Am. Chem. Soc.* **1960**, *82*, 2601–2605. (b) Battersby, A. R.; Leeper, F. J. *Chem. Rev.* **1990**, *90*, 1261–1274.

(57) Hydrogenation over palladium catalysts can also be used to generate porphyrinogens, thereby avoiding the use of mercury, but this method is not compatible with the presence of vinyl side chains. See: Bergonia, H. A.; Phillips, J. D.; Kushner, J. P. *Anal. Biochem.* **2009**, *384*, 74–78.

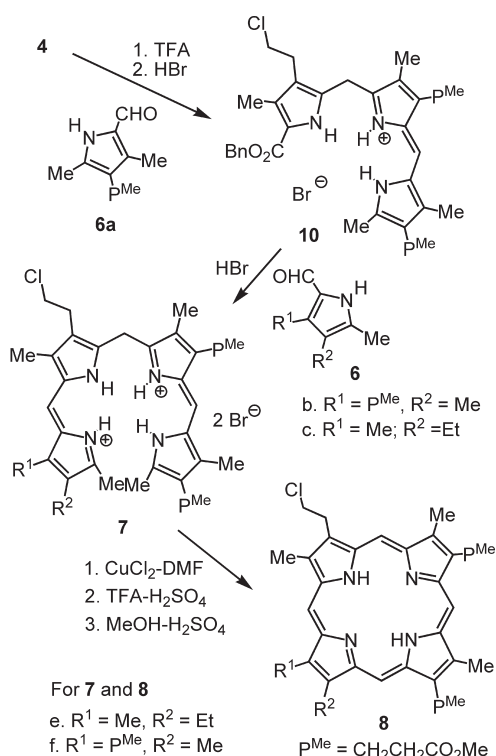
Scheme 7



Scheme 8



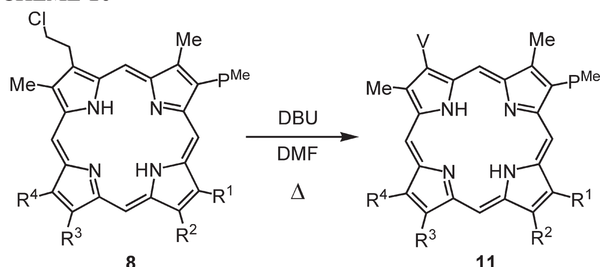
Scheme 9



and the 13- and 17-ethyl analogues of harderoporphyrin-III were not previously known. In order to simplify these investigations, all six targeted porphyrins were prepared from a single dipyrrylmethane **4** via *a*,*c*-biladiene intermediates (Schemes 7−9). The vinyl unit must be installed after the porphyrin macrocycle has been generated due to the highly reactive nature of vinylic pyrroles, and this was accomplished by using a chloroethyl side chain as a protected vinyl moiety.[10] Dipyrrylmethane **4**, which was prepared by a literature procedure,[10] has three types of ester units. The benzyl ester is easily hydrogenolyzed in quantitative yield to give the corresponding carboxylic acid **5** (Scheme 7). Treatment with TFA cleaves the *tert*-butyl ester and decarboxylates both of the terminal units, and subsequent reaction with 2 equiv of pyrrole aldehydes **6a** or **6b** gave the corresponding *a*,*c*-biladienes **7a** and **7b**, respectively. These open-chain tetrapyrrolic salts were precipitated with ether to give red powders in 62−77% yield. Cyclization with copper(II) chloride in DMF at room temperature,[58] followed by demetalation with 15% sulfuric acid−TFA and reesterification with 5% H_2SO_4−methanol, gave the chloroethyl precursors

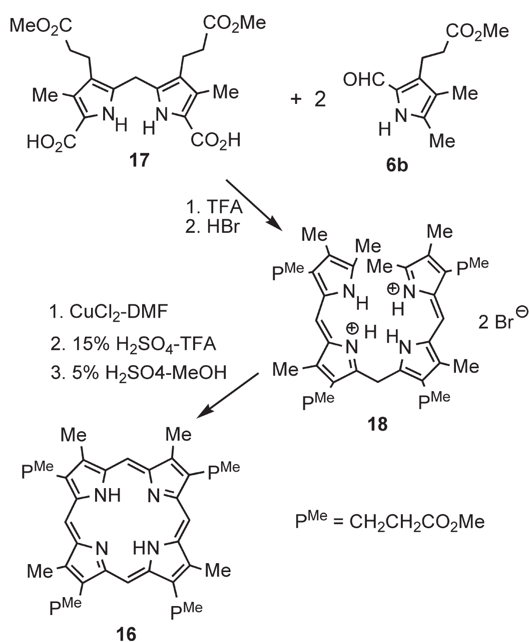
(58) Smith, K. M.; Minnetian, O. M. *J. Chem. Soc., Perkin Trans. 1* **1986**, 277–280.

SCHEME 10



a. Harderoporphyrin-III $R^1 = R^4 = Me$; $R^2 = R^3 = P^{Me}$
b. Isoharderoporphyrin $R^1 = R^4 = P^{Me}$; $R^2 = R^3 = Me$
c. Harderoporphyrin-VII $R^1 = R^3 = P^{Me}$; $R^2 = R^4 = Me$
d. 13Et Hardero $R^1 = R^4 = Me$; $R^2 = Et$; $R^3 = P^{Me}$
e. 17Et Hardero $R^1 = R^4 = Me$; $R^2 = P^{Me}$; $R^3 = Et$
f. Harderoporphyrin-I $R^1 = R^3 = Me$; $R^2 = R^4 = P^{Me}$

SCHEME 11



to harderoporphyrin-III and isoharderoporphyrin, **8a** and **8b**, respectively, in 54% and 38% yields.

The remaining porphyrins had to be prepared via stepwise routes.[59,60] Dipyrrylmethane carboxylic acid **5** was reacted with 1 equiv of pyrrole aldehyde **6a** in the presence of *p*-toluenesulfonic acid, followed by brief treatment with anhydrous HBr, to give the tripyrrene *tert*-butyl ester **9** in 52% yield (Scheme 8). Subsequent treatment with TFA and reaction of formylpyrroles **6b** and **6c** gave the related *a*,*c*-biladienes **8c** and **8d** in 74–81% yield. Cyclization with $CuCl_2$ in DMF, followed by demetalation and reesterification, gave the chloroethylporphyrins **8c** and **8d** in 59% and 58% yields, respectively. The remaining two porphyrins were prepared by an alternative tripyrrene strategy (Scheme 9).[60] The mixed ester dipyrrylmethane **4** was treated with TFA to

(59) Baptista de Almeida, J. A. P.; Kenner, G. W.; Rimmer, R.; Smith, K. M. *Tetrahedron* **1976**, *32*, 1793–1799.
(60) Smith, K. M.; Craig, G. W. *J. Org. Chem.* **1983**, *48*, 4302–4306.

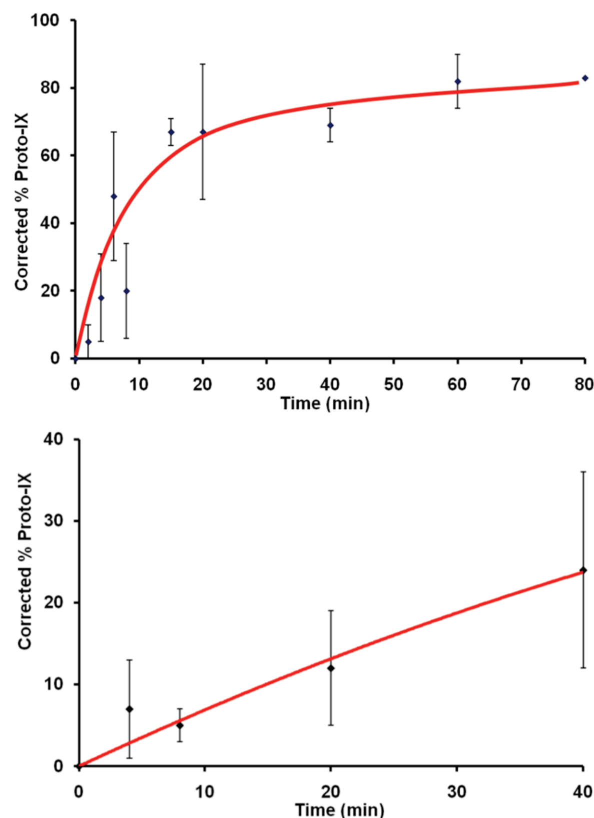


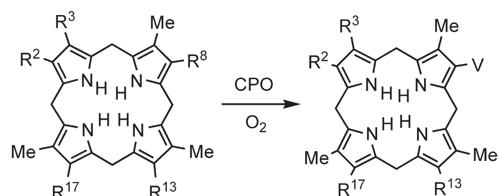
FIGURE 4. Time-course experiments for incubations of harderoporphyrinogen-III (upper trace) and isoharderoporphyrinogen (lower trace) with chicken red cell hemolysates at 37 °C showing the percent conversion of the substrate to protoporphyrin-IX. These values were corrected to take into account the presence of endogenous protoporphyrin-IX that is present in these crude enzyme preparations by the method described in ref 42.

cleave the *tert*-butyl ester and reacted with pyrrole aldehyde **6a** and HBr to give the tripyrrene benzyl ester **10** in 75% yield. Further treatment with HBr in TFA for 6 h cleaved the benzyl ester, and subsequent condensation with pyrrole aldehyde **6c** gave *a*,*c*-biladiene **7e** in 66% yield. Similarly, reaction with pyrrole aldehyde **6b** gave the *a*,*c*-biladiene precursor **7f** to harderoporphyrin-I in 65% yield. Copper(II) chloride mediated cyclization, demetalation, and reesterification gave porphyrins **8e** and **8f** in 66% and 70% yields, respectively. The vinyl groups were introduced by base catalyzed dehydrohalogenation of the chloroethyl side chains (Scheme 10). In earlier work,[10] we had carried out this transformation with KOH-pyridine based on a literature procedure,[61] but these conditions gave incomplete conversions for the current series. This difficulty was easily overcome by heating the chloroethylporphyrins **8** with DBU in DMF for 1 h. Following chromatography and recrystallization, the vinyl porphyrins **11** were isolated in 75–80% yield.

In order to completely assess these substrates, samples of coproporphyrin-IV (**16**) were also required. This was prepared in two steps from dipyrrylmethane dicarboxylic acid **17**[62] (Scheme 11). Reaction of **17** with pyrrole aldehyde **6b** in

(61) Clezy, P. S.; Fookes, C. J. R. *Aust. J. Chem.* **1977**, *30*, 217–220.
(62) Lash, T. D.; Armiger, Y. L. S.-T. *J. Heterocycl. Chem.* **1991**, *28*, 965–970.

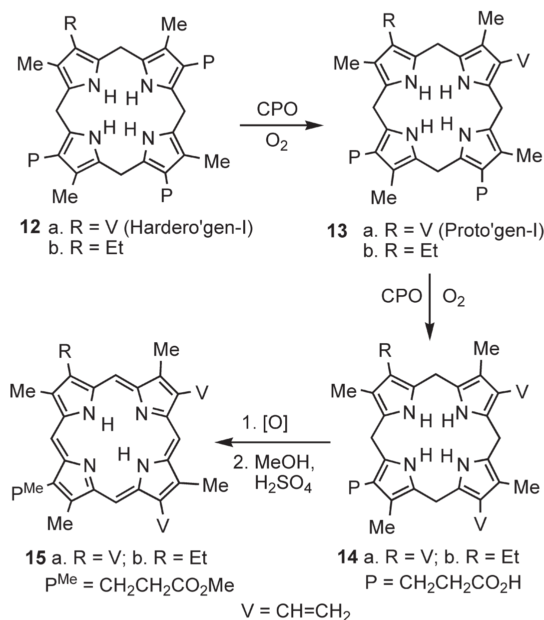
SCHEME 12



Hardero'gen-III R^2 = Me; R^3 = V; R^8 = R^{13} = R^{17} = P (good substrate)
Isohardero'gen R^2 = Me; R^8 = V; R^3 = R^{13} = R^{17} = P (poor substrate)
Hardero'gen-VII R^2 = V; R^3 = Me; R^8 = R^{13} = R^{17} = P (good substrate)
2e (13Et) R^2 = Me; R^3 = V; R^8 = R^{17} = P; R^{13} = Et (non-substrate)
2f (17Et) R^2 = Me; R^3 = V; R^8 = R^{13} = P; R^{17} = Et (good substrate)

P = $CH_2CH_2CO_2H$, V = $CH=CH_2$

SCHEME 13



the presence of TFA and HBr gave the *a*,*c*-biladiene **18**, and this was cyclized with $CuCl_2$ in DMF, demetalated, and reesterified to give coproporphyrin-IV tetramethyl ester in 51% yield.[63]

The vinylporphyrins were hydrolyzed with 25% hydrochloric acid and reduced to the corresponding porphyrinogen carboxylic acids with 3% sodium amalgam as described previously.[10] These were then incubated at 37 °C with chicken red cell hemolysates (CRH), which act as an active source of CPO.[10] Following the incubation, the products were extracted as the corresponding porphyrins and esterified prior to analysis. Product formation was assessed by TLC and normal-phase HPLC. Although CRH is an excellent source of CPO, it is contaminated by small amounts of protoporphyrin-IX and the data must be corrected for this contaminant. In addition, the porphyrinogens are air sensitive, but oxygen must be present for enzyme activity. These factors lead to some variations that can produce large error bars. However, the experiments were usually conducted in triplicate and show reasonably reproducible results. As expected, hardero'gen-III was an excellent substrate (Figure 4) and was rapidly converted to protoporphyrin-IX (Scheme 12). Isoharderporphyrin is a much poorer substrate, but our model for enzyme activity suggests that this porphyrinogen should not be a substrate at all as it does not possess the usual sequence of substituents R Me-P Me-P. However, careful analysis of the data showed that isohardero'gen is indeed a substrate for CPO as has been noted previously in the literature.[30] The presence of endogenous protoporphyrin-IX does make the results a little ambiguous, but preliminary results using human recombinant CPO,[37] which does not have this contaminant, also show the formation of protoporphyrin-IX, and there can be no doubt that isohardero'gen is a substrate for this enzyme. Hardero'gen-VII is a good substrate for CPO and is metabolized at a comparable rate to hardero'gen-III, but the 13-ethyl analogue of hardero'gen-III **2e** showed no conversion to the divinyl product (Scheme 12), confirming earlier results for tripropionate porphyrinogens.[41,42] However, the 17-ethyl analogue was an excellent substrate, again as had been expected. In earlier work, tripropionate porphyrinogen **1e** had been incubated with CRH to give the 13-ethylporphyrinogen **2e** (Scheme 5), and this was further characterized by NMR spectroscopy for the related porphyrin dimethyl ester. Synthetic 13-ethylharderoporphyrin **11d** gave a virtually identical 300 MHz proton NMR spectrum to the product isolated from those incubation studies[41] and was easily distinguishable from the proton NMR spectrum for the isomeric 17-ethylharderoporphyrin **11e** (see the Supporting Information). Hence, the synthetic samples further confirm the structure of this incubation product.

(63) The biochemical studies conducted on copro'gen-IV are reported in ref 37.

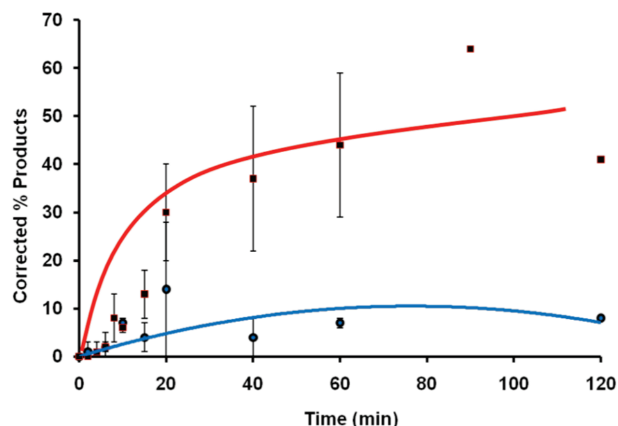


FIGURE 5. Time-course experiments for incubations of harderoporphyrinogen-I with chicken red cell hemolysates showing the formation of protoporphyrinogen-I (blue line) and trivinylporphyrinogen **14a** (red line) over a 2 h incubation period at 37 °C.

As expected, hardero'gen-I (**12a**) also proved to be a good substrate for CPO (Scheme 13, Figure 5). Following incubations with CRH, extraction, and esterification, the products were analyzed by HPLC (Figure 6). These traces showed the formation of two products with retention times that were consistent with di- (peak B) and monoesters (peak C), respectively. Although **12a** is a non-natural type I porphyrinogen, it has the correct sequence of peripheral substituents for binding to CPO. Oxidative decarboxylation would then afford protoporphyrinogen-I (**13a**), but this species also has

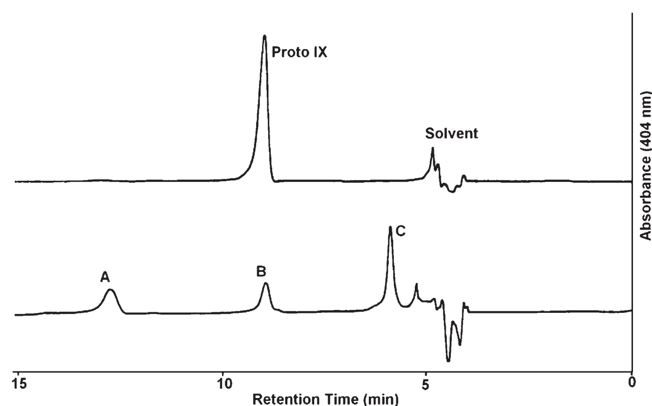


FIGURE 6. HPLC traces showing the porphyrin methyl ester products for a 20 min incubation of harderoporphyrinogen-I with CRH and a standard chromatogram for protoporphyrin-IX. The HPLC analysis was performed on a normal-phase 5 μm Partisil colum (250 × 4.6 mm), using a 20 μL injection loop, with a mobile phase of 35/65 v/v ethyl acetate–cyclohexane. Peak A = harderoporphyrin-I; peak B = protoporphyrin-I; peak C = trivinylporphyrin **15a**.

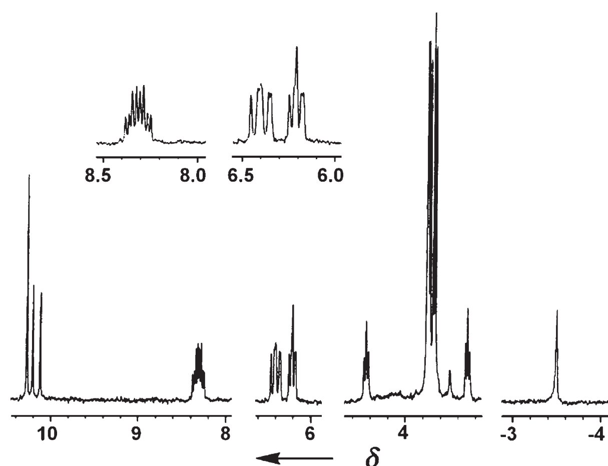


FIGURE 7. 300 MHz proton NMR spectrum of the trivinylporphyrin methyl ester derived from incubations of harderoporphyrinogen-I with chicken red cell hemolysates.

the correct sequence of substituents (V Me-P Me-P) and is apparently further processed to give the trivinylporphyrinogen **14a**. This observation provides striking evidence in support of our substrate binding model. Protoporphyrinogen-I does not accumulate in these incubation studies and probably remains associated with CPO so that it is converted directly into **14a**. A preparative study was conducted where **12a** was incubated with CRH and the trivinylporphyrin product **15a** derived from porphyrinogen **13a** was isolated and characterized by FAB MS and proton NMR spectroscopy. High-resolution FAB MS gave an [M + H] peak at m/z 531.2758 which corresponds to the expected formula $C_{34}H_{34}N_4O_2$ + H. The proton NMR spectrum (Figure 7) was also in full agreement with the proposed trivinylporphyrin structure **15a**, showing three singlets for the *meso*-protons at 10.28 (2H), 10.21 (1H), and 10.12 ppm (1H), and three 3H multiplets for the vinyl units at 8.24–8.38, 6.35–6.45, and 6.17–6.25 ppm. The chloroethyl precursor **8f** was also hydrolyzed and reduced with 3% sodium amalgam to give the 3-ethylporphyrinogen **12b**. This dihydroharderoporphyrin was also a good substrate and gave a monopropionate product **14b** (Scheme 13). The metabolite was characterized as the porphyrin methyl ester **15b** by HR FAB MS and proton NMR spectroscopy (see the Supporting Information).

Conclusions

A series of monovinylic porphyrins have been synthesized, specifically harderoporphyrin-III, three isomeric compounds, and two ethyl-substituted analogues. These porphyrins were converted into the corresponding porphyrinogen carboxylic acids and used to probe the activity of coproporphyrinogen oxidase (CPO). The results from these studies confirm that a specific sequence of peripheral substituents is generally required for substrate binding and metabolism, although isoharderoporphyrinogen lacks this sequence and can still act as a poor substrate for this enzyme. Nevertheless, the 17-ethyl analogue of harderoporphyrinogen-III has the required sequence and is a good substrate, while its 13-ethyl isomer, which lacks the correct sequence, is not metabolized. Harderoporphyrinogen-I is also a good substrate for CPO, but in this case the first formed product is also a substrate, and this allows further conversion to an unusual trivinylporphyrinogen product. These results help to clarify the binding requirements for CPO and will allow further investigations into the activity of this important enzyme in the heme biosynthetic pathway.

Experimental Section

Benzyl 3-(2-Chloroethyl)-8,13-bis(2-methoxycarbonylethyl)-2,7,12,14-tetramethyl-5,16-dihydrotripyrrin-1-carboxylate Hydrobromide (10). Dipyrrylmethane **4**[10] (1.00 g, 1.80 mmol) was treated with trifluoroacetic acid (6.3 mL) with stirring under a nitrogen atmosphere at ambient temperature for 5 min. A solution of pyrrole aldehyde **6a**[9] (0.377 g, 1.80 mmol) in methanol (40 mL) was added all at once, and the brownish orange solution was stirred further for 1.5 h. Commercially available 30% HBr–acetic acid (8 drops) was added, followed by dropwise addition of ether (80 mL), and the mixture was stirred for a further 15 min. The orange precipitate was filtered, washed thoroughly with ether, and dried in vacuo overnight to give the title tripyrrene hydrobromide (0.962 g, 1.32 mmol, 75%) as orange crystals: mp 177.5–178.5 °C; UV–vis ($CHCl_3$) λ_{max} ($\log_{10} \varepsilon$) 493 nm (4.90); ^{1}H NMR ($CDCl_3$) δ 2.07 (3H, s), 2.26 (3H, s), 2.35 (3H, s), 2.46–2.54 (4H, 2 overlapping triplets), 2.66 (3H, br s), 2.76 (2H, t, J = 7.6 Hz), 2.91 (2H, t, J = 7.8 Hz), 2.96 (2H, t, J = 7.6 Hz), 3.37 (2H, t, J = 7.8 Hz), 3.61 (3H, s), 3.67 (3H, s), 4.33 (2H, br s), 5.31 (2H, s), 7.25–7.3 (3H, m), 7.31 (1H, s), 7.49 (2H, d, J = 7.2 Hz), 10.71 (1H, br s), 13.11 (1H, br s), 13.15 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 9.3, 10.4, 10.7, 13.3, 19.5, 20.3, 23.3, 28.5, 33.9, 34.7, 44.1, 52.0, 52.1, 65.6, 118.4, 119.1, 121.1, 123.1, 125.7, 127.3, 128.0, 128.4, 128.6, 137.0, 144.4, 144.8, 150.6, 157.4, 161.1, 172.7. Anal. Calcd for $C_{36}H_{43}N_3O_6ClBr.H_2O$: C, 57.87; H, 6.07; N, 5.62. Found: C, 57.98; H, 5.76; N, 5.47.

8-(2-Chloroethyl)-3,13,18-tris(2-methoxycarbonylethyl)-1,2,7,-12,17,19-hexamethyl-10,23-dihydrobilin Dihydrobromide (7f). Tripyrrene **10** (124 mg, 0.17 mmol) was stirred with a mixture of 30% HBr–acetic acid (0.5 mL) and trifluoroacetic acid (2.5 mL) at room temperature for 6 h. A solution of pyrrole aldehyde **6b**[9] (36 mg, 0.17 mmol) was added all at once to the brownish orange solution, which immediately turned reddish orange, and stirred for 30 min. Ether (30 mL) was added rapidly but dropwise, and stirring was continued for 15 min. The resulting

precipitate was suction filtered, washed thoroughly with ether, and dried in vacuo overnight to give the *a,c*-biladiene (94 mg, 0.11 mmol, 65%) as a red powder: mp 176.5−177 °C; UV−vis ($CHCl_3$) λ_{max} ($\log_{10} \varepsilon$) 455 (4.42), 524 nm (5.32); ^{1}H NMR ($CDCl_3$) δ 1.99 (3H, s), 2.03 (3H, s), 2.33 (3H, s), 2.37 (3H, s), 2.49 (4H, t, J = 7.3 Hz), 2.56 (2H, t, J = 7.2 Hz), 2.69 (3H, s), 2.72 (3H, s), 2.77 (2H, t, J = 7.5 Hz), 2.92−3.06 (8H, m), 3.60 (3H, s), 3.64 (3H, s), 3.68 (3H, s), 5.21 (2H, s), 7.30 (1H, s), 7.34 (1H, s), 13.31 (1H, br s), 13.35 (1H, br s), 13.46 (1H, br s), 13.48 (1H, br s); ^{1}H NMR ($CDCl_3$) δ 9.1, 9.5, 10.4, 10.7, 13.3, 13.4, 19.6, 20.1, 20.3, 25.9, 27.5, 33.9, 34.7, 34.8, 43.7, 52.0, 52.1, 52.2, 121.1, 121.3, 125.1, 121.56, 121.63, 126.1, 126.7, 127.9, 128.4, 142.1, 144.2, 144.9, 145.6, 148.5, 149.4, 157.3, 157.7, 172.6, 172.7, 172.8. Anal. Calcd for $C_{39}H_{51}Br_2ClN_4O_6 \cdot {}^1/_2H_2O$: C, 53.47; H, 5.98; N, 6.39. Found: C, 53.31; H, 5.76; N, 6.26.

3-(2-Chloroethyl)-8,13,18-tris(2-methoxycarbonylethyl)-2,7,12,-17-tetramethylporphyrin (8f). *a,c*-Biladiene dihydrobromide **7f** (460 mg, 0.53 mmol) was added to a stirred solution of copper(II) chloride (1.31 g) in DMF (195 mL), and the resulting mixture was stirred in dark for 2 h. The mixture was diluted with dichloromethane (250 mL) and washed with water (3 × 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 a vacuum pump was used to remove any remaining DMF. The solid residue was taken up in 15% v/v sulfuric acid−TFA (50 mL) and stirred in the dark at room temperature for 45 min. The reaction mixture was diluted with dichloromethane (250 mL) and washed with water (2 × 250 mL) and 5% aqueous sodium bicarbonate solution (250 mL). The aqueous layers were back-extracted with dichloromethane, the combined organic layers were dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was dissolved in 5% sulfuric acid−methanol (50 mL) and stirred at room temperature in the dark overnight. The mixture was diluted with dichloromethane and washed with water and then with 5% aqueous sodium bicarbonate solution. The aqueous layers were back-extracted with dichloromethane at each stage, the combined organic layers were dried over sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was chromatographed on a grade 3 alumina column, eluting with dichloromethane. A dark violet product fraction was collected, the solvent was evaporated under reduced pressure, and the residue recrystallized from chloroform−methanol to give the chloroethylporphyrin (254 mg, 0.37 mmol, 70%) as fluffy maroon crystals: mp 243−244 °C; UV−vis (1% $Et_3N-CHCl_3$) λ_{max} ($\log_{10} \varepsilon$) 401 (5.26), 499 (4.25), 533 (4.13), 568 (4.01), 622 nm (3.92); UV−vis (1% TFA−$CHCl_3$): λ_{max} ($\log_{10}\varepsilon$) 408 (5.55), 551 (4.29), 593 nm (4.02); ^{1}H NMR ($CDCl_3$) δ −3.76 (2H, br s), 3.28 (6H, t, J = 7.8 Hz), 3.65 (3H, s), 3.670 (3H, s), 3.672 (3H, s), 3.686 (6H, s), 3.688 (3H, s), 3.693 (3H, s), 4.33 (2H, t, J = 7.6 Hz), 4.39−4.52 (6H, m), 4.54 (2H, t, J = 8.0 Hz), 10.03 (1H, s), 10.09 (2H, s), 10.11 (1H, s); ^{1}H NMR (TFA−$CDCl_3$) δ −3.55 (4H, br s), 3.16 (6H, t, J = 7.4 Hz), 3.68 (9H, s), 3.69 (3H, s), 3.697 (3H, s), 3.701 (3H, s), 3.73 (3H, s), 4.20 (2H, t, J = 7.2 Hz), 4.45−4.50 (6H, m), 4.57 (2H, t, J = 7.2 Hz), 10.69 (1H, s), 10.81 (1H, s), 10.82 (1H, s), 10.84 (1H, s); ^{13}C NMR (TFA−$CDCl_3$) δ 12.1, 12.2, 12.3, 22.0, 30.1, 35.7, 44.1, 52.8, 99.3, 99.4, 99.5, 99.6, 138.1, 139.3, 139.5, 140.2, 140.4, 141.8, 141.9, 142.1, 142.4, 142.5, 142.6, 174.8. Anal. Calcd for $C_{38}H_{43}ClN_4O_6$: C, 66.41; H, 6.31; N, 8.15. Found: C, 66.04; H, 6.25; N, 8.00.

8,13,18-Tris(2-methoxycarbonylethyl)-2,7,12,17-tetramethyl-3-vinylporphyrin (11f; Harderoporphyrin-I Trimethyl Ester). DBU (5 drops) was added to a solution of chloroethylporphyrin **8f** (40 mg, 0.058 mmol) in DMF (40 mL), and the mixture was refluxed with stirring under nitrogen for 1 h. The solution was cooled, diluted with dichloromethane, and washed with 5% hydrochloric acid, 5% aqueous sodium bicarbonate solution, and water. The solvent was evaporated under reduced pressure, and the purple residue was purified by chromatography on a grade 3 alumina column eluting with dichloromethane. Recrystallization from chloroform−methanol gave harderoporphyrin-I trimethyl ester (29 mg, 0.045 mmol, 77%) as small purple needles: mp 178.5−180 °C; UV−vis (1% $Et_3N-CHCl_3$) λ_{max} ($\log_{10} \varepsilon$) 403 (5.20), 502 (4.17), 538 (4.10), 572 (3.94), 626 nm (3.80); UV−vis (1% TFA−$CHCl_3$) λ_{max} ($\log_{10} \varepsilon$) 408 (5.55), 551 (4.29), 593 nm (4.02); ^{1}H NMR ($CDCl_3$) δ −3.68 (2H, br s), 3.24−3.32 (6H, m), 3.64 (3H, s), 3.66 (3H, s), 3.68 (6H, s), 3.69 (3H, s), 3.70 (3H, s), 3.74 (3H, s), 4.37−4.48 (6H, m), 6.18 (1H, dd, J = 1.8, 11.1 Hz), 6.37 (1H, dd, J = 1.8, 17.4 Hz), 8.30 (1H, dd, J = 11.1, 17.4 Hz), 10.07 (2H, s), 10.14 (1H, s), 10.23 (1H, s); ^{1}H NMR (TFA−$CDCl_3$) δ −3.47 (3H, br s), −3.35 (1H, br s), 3.12−3.18 (6H, m), 3.647 (3H, s), 3.653 (3H, s), 3.66 (3H, s), 3.68 (3H, s), 3.69 (6H, s), 3.75 (3H, s), 4.42−4.49 (6H, m), 6.30 (1H, d, J = 17.6 Hz), 6.49 (1H, d, J = 11.6 Hz), 8.15 (1H, dd, J = 11.6, 17.6 Hz), 10.69 (1H, s), 10.78 (2H, s), 10.84 (1H, s); ^{13}C NMR (TFA−$CDCl_3$) δ 12.0, 12.1, 12.7, 21.9, 35.6, 52.6, 99.1, 99.2, 99.8, 99.9, 127.9, 138.4, 138.5, 139.1, 139.2, 139.3, 140.1, 140.3, 141.0, 141.7, 141.8, 142.1, 142.5, 142.6, 174.4, 174.5; EIMS (70 eV) m/z (relative intensity) 652 (1.6), 651 (4.7), 650 (10.2, M^+), 578 (1.6), 577 (21, $[M - CH_2CO_2CH_3]^+$); HRMS (EI) m/z calcd for $C_{38}H_{42}N_4O_6$ 650.3104, found 650.3102. Anal. Calcd for $C_{38}H_{42}N_4O_6 \cdot {}^1/_{10}CHCl_3$: C, 69.05; H, 6.40; N, 8.45. Found: C, 68.76; H, 6.36; N, 8.31.

Enzyme Incubation Studies. Enzyme incubations and analyses of metabolic products were carried out as described previously.[10] HPLC analyses were performed using normal-phase columns (5 m Partisil silica, Alltech) eluting with appropriate ratios of ethyl acetate and cyclohexane. Kinetic data are reported as mean ± standard deviation for three replicate experiments, except in the case of isohardero'gen where only two were performed, and compared statistically using analysis of variance (ANOVA) following Fisher's LSD post test. Values are considered different at $p < 0.05$.

Porphyrins Isolated from Preparative Enzymic Studies. These metabolites were obtained by reducing the tricarboxylic acids derived from **11f** and **8f**, respectively, with 3% sodium amalgam and incubating the resulting porphyrinogens with chicken red cell hemolysates. The products were purified as their methyl esters by flash chromatography, eluting with 10% ethyl acetate−toluene, and characterized by FAB MS and 300 MHz proton NMR spectroscopy.

18-(2-Methoxycarbonylethyl)-2,7,12,17-tetramethyl-3,8,13-trivinylporphyrin (15a): ^{1}H NMR ($CDCl_3$) δ −3.49 (2H, br s), 3.29 (2H, t, J = 7.6 Hz), 3.66 (3H, s), 3.68 (3H, s), 3.72 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 4.44 (2H, t, J = 7.6 Hz), 6.17−6.25 (3H, m), 6.35−6.45 (3H, m), 8.24−8.38 (3H, m), 10.12 (1H, s), 10.21 (1H, s), 10.28 (2H, s); HRMS (FAB) m/z calcd for $C_{34}H_{34}N_4O_2$ + H 531.2760, found 531.2758.

3-Ethyl-18-(2-methoxycarbonylethyl)-2,7,12,17-tetramethyl-8,13-divinylporphyrin (15b): ^{1}H NMR ($CDCl_3$) δ −3.5 (2H, br s), 1.88 (3H, t, J = 7.5 Hz), 3.27 (2H, t, J = 7.6 Hz), 3.63 (3H, s), 3.65 (3H, s), 3.67 (3H, s), 3.74 (3H, s), 3.75 (3H, s), 4.11 (2H, q, J = 7.5 Hz), 4.40 (2H, t, J = 7.6 Hz), 6.18−6.23 (2H, m), 6.38 (2H, d, J = 17.7 Hz), 8.31 (2H, m), 10.05 (1H, s), 10.14 (1H, s), 10.22 (1H, s), 10.29 (1H, s); HRMS (FAB) m/z calcd for $C_{34}H_{36}N_4O_2$ + H 533.2916, found 533.2917.

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Supporting Information Available: Full experimental details, ^{1}H NMR and ^{13}C NMR spectra for selected compounds, and selected MS and HPLC data. This material is available free of charge via the Internet at http://pubs.acs.org.