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Total Synthesis of Hematoporphyrin and Protoporphyrin: A Conceptually New Approach

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Abstract:

The total synthesis of protoporphyrin IX and its disodium salt using a new alternative method to the classical MacDonald condensation is reported. The key step is the reaction of the new unsymmetrical diiodo dipyrrylmethane 1 with the known dipyrrylmethane 2. Coupling of the two fragments leads directly to porphyrin 3 without the need of an oxidizing agent. The new methodology is well suited for the synthesis of protoporphyrin IX derivatives on a multi 100 g scale in good quality without the need for chromatography. Furthermore, these preparations are completely free of any contaminant of animal origin, which represents a real improvement in the manufacturing of protoporphyrin IX derivatives.

Introduction

Protoporphyrin IX (Figure 1) is most commonly prepared by semisynthesis starting from hemin, which in turn is isolated from ox or pork blood.[1,2] For medical applications, e.g., in cell culture media, this poses the problem of impurities of animal origin that may be present, in particular of agents in connection with transmissible spongiform encephalopathy. In order to meet the guidelines of the regulatory agencies,[3] it was necessary to provide a completely synthetic preparation process for the preparation of protoporphyrin IX which uses only products of synthetic origin.

Here, we describe the total synthesis of protoporphyrin IX and its disodium salt using a novel variant of the MacDonald condensation method. The key step is the reaction of the new unsymmetrical diiododipyrrylmethane **1** with the known dipyrrylmethane **2** (Scheme 1).[4] The two fragments are coupled without the presence of a metal leading directly to porphyrin **3** without the need of an oxidizing agent. The new methodology is well suited for the synthesis of protoporphyrin IX on a multi 100 g scale in good quality without the need for chromatography.

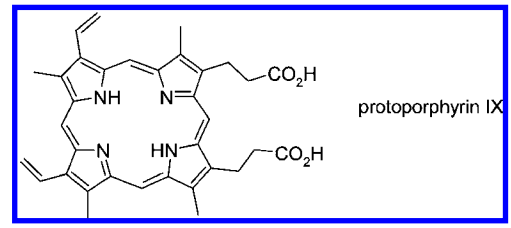

Figure 1

Synthetic Concept. The classical MacDonald condensation where a dipyrrylmethane unit is reacted with a dipyrrylmethane dialdehyde has been widely applied for the preparation of a variety of unsymmetrically substituted porphyrins.[5] The primary product is a dihydroporphin which must then be oxidized to obtain the corresponding porphyrin using either air[5] or a quinone such as DDQ.[6] In many cases, the oxidation step is rather problematic since the dihydroporphin is quite unstable and not easy to detect. Furthermore, the reduced quinone is difficult to remove from the reaction mixture requiring a chromatographic separation. For these reason, scale up problems are unavoidable and, indeed, literature preparations are usually described in the mg range.

We reasoned that in order to avoid the formation of the dihydroporphin, one of the two dipyrrylmethanes should either be in a higher oxidation state or carry a nucleofuge which can be eliminated to form the required additional CC double bond. We decided to prepare the diiodo derivative **1** and to react it under MacDonald conditions with dipyrrylmethane dialdehyde **2** (Scheme 1). To our delight and surprise, we directly obtained the desired porphyrin **3** in good yields. We postulate that the primary condensation product is intermediate **A** which spontaneously eliminates water as well as one mol of HI to give the iodinated porphyrin **B** which then is reduced by the HI formed in the first step to give **3** and I_2.[7] We consider the elimination of 2 mol of HI improbable since a rather thermodynamically unfavorable dehydroporphyrin would be formed.

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(1) The original procedure was first described by Schalfejew, M. *Hoppe Seyler's Z.* **1900**, *30*, 390. *Encyklopädie der technischen Chemie (Ullmann's Encyclopedia of Industrial Chemistry)*, IV ed.; VCH: Weinheim, 1976; Vol. 11, p 129. An improved procedure is described: Schulze, H. (BASF). U.S. Patent 4,761,472, 1988. About 3−4 g of heme are obtained from 1 l of oxblood.

(2) Jackson, A. H.; Rao, K. R.; Wilkins, M. *J. Chem. Soc., Perkin Trans. 1* **1987**, 307. Chen, W.; Yang, H.; Xu, D. *Zhongguo Yiyao Gongye Zazhi* **2000**, *31*, 293. *CAN 134*, 193262.

(3) Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products. *Official Journal of the European Union*, C24/11; January 28, 2004; p 6. *Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products*, EMEA 410/01/Rev. 2; Committee for Medicinal Products (CHMP), European Medicines Agency (EMEA): London, 2004.

(4) Chong, R.; Clezy, P. S.; Liepa, A. J.; Nichol, A. W. *Aust. J. Chem.* **1969**, *22*, 229. Jackson, A. H.; Pandey, R. K. *J. Chem. Soc., Perkin Trans. 1* **1987**, 299.

(5) Arenault, G. P.; Bullock, E.; MacDonald, S. F. *J. Am. Chem. Soc.* **1960**, *82*, 4384. Chakrabarty, M. *J. Ind. Chem. Soc* **2001**, *78*, 761. Clezy, P. S.; Diakiw, V. *Aust. J. Chem.* **1975**, *28*, 2703.

(6) Lee, C.-H.; Li, F.; Iwamoto, K.; Dadok, J.; Bothner-By, A. A.; Lindsay, J. S. *Tetrahedron* **1995**, *51*, 11645.

(7) Indeed, I_2 could be observed when the reaction solution was heated in the Rotovap. Furthermore, small amounts of a compound could be isolated via chromatography which showed the appropriate mass corresponding to intermediate **B** and reacted to give **3** in the presence of HI.

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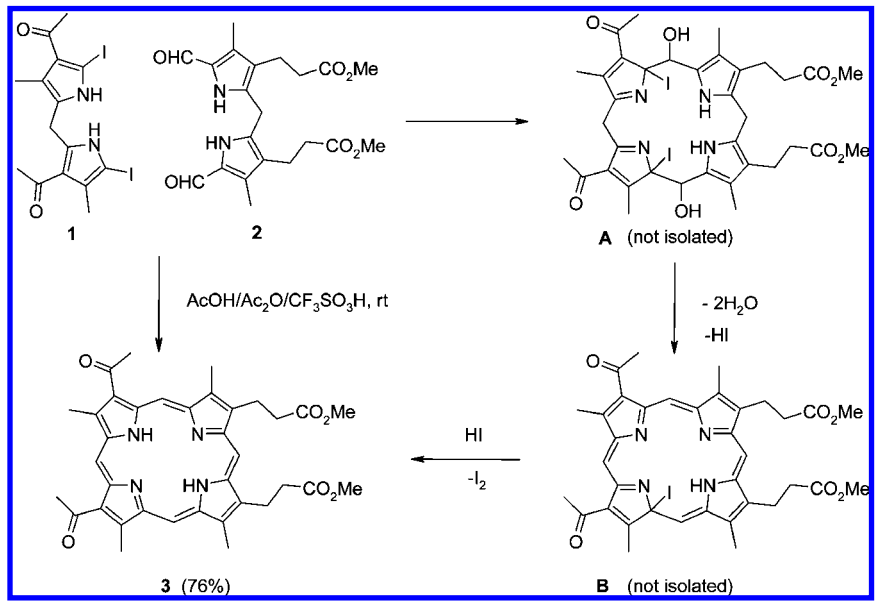
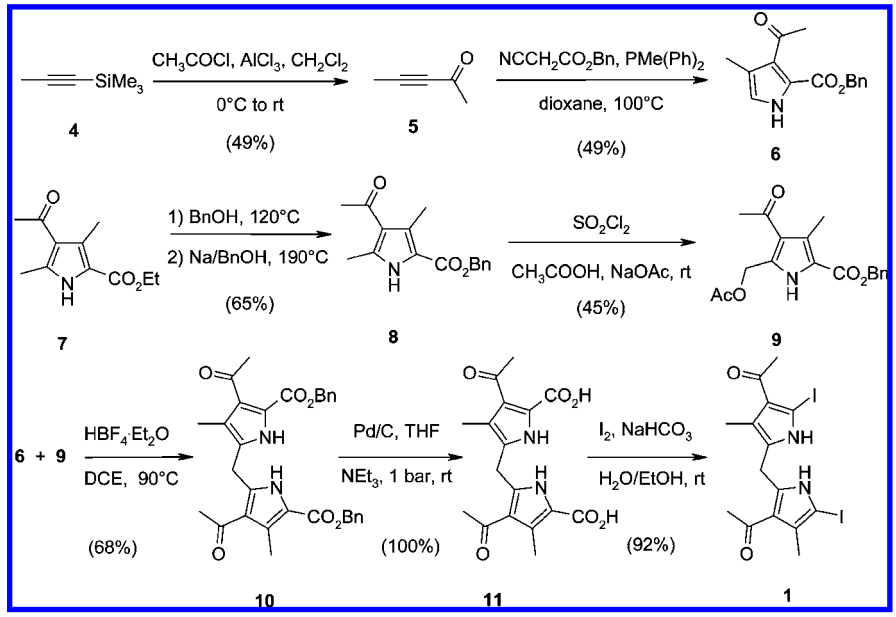
Scheme 1



Scheme 2

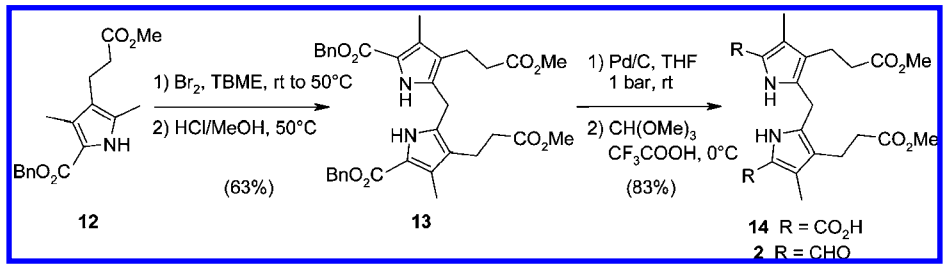


Synthetic Details. The synthesis of the new diiodo dipyrrylmethane **1** was carried out as depicted in Scheme 2. The partially optimized synthesis which delivered the desired intermediate in an acceptable overall yield, starts with the silylated propyne **4** which is reacted with acetyl chloride in presence of $AlCl_3$ to give 3-pentyne-2-one **5**. The phosphine catalyzed reaction of **5** with benzyl isocyanoacetate gave the first pyrrole unit **6** which was obtained in pure form after chromatography in moderate yield. The second pyrrole moiety **9** was prepared starting from the commercially available pyrrole ethyl ester **7** which was first transformed to the corresponding benzyl ester **8** with Na/BnOH at 190 °C and then acetoxylated in presence of sulfuryl chloride to give **9** in moderate yield. The condensation of the two pyrrolo units **6** and **9** catalyzed by HBF_4 gave the desired dipyrrylmethane **10** in an acceptable yield of 68%. After removal of the benzyl groups via hydrogenolysis with Pd/C to give the diacid **11**, the novel diiodo dipyrrylmethane **1** was obtained in excellent yield and high purity as light red crystals.

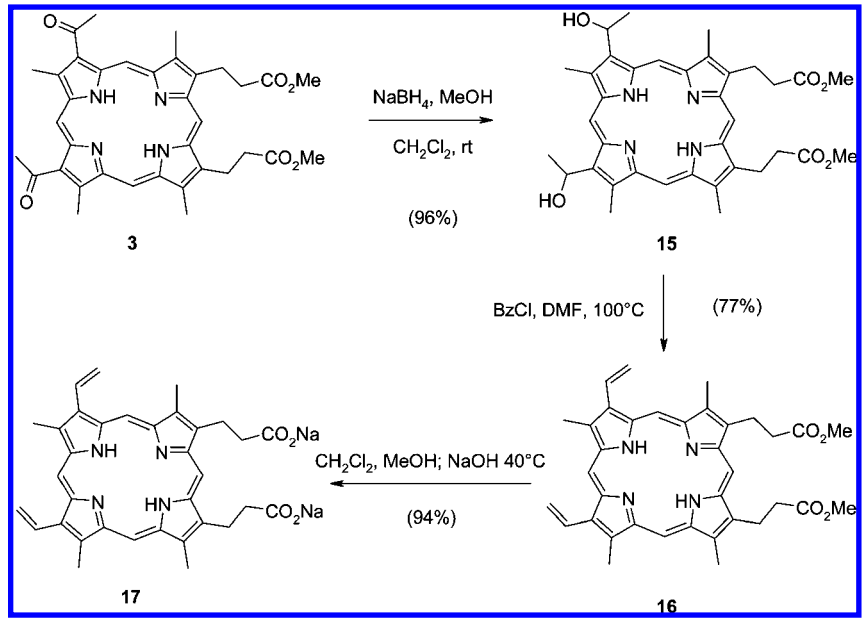
The synthesis of the second dipyrrylmethane moiety **2** was carried out as depicted in Scheme 3. Starting from commercially available **12** which was brominated selectively and then condensed with the loss of a C_1 unit to give dipyrrylmethane **13** in good yield. Dialdehyde **2** was then obtained in excellent yield via debenzylation with Pd/C to give **14**, followed by reduction with trimethyl orthoformate in presence of trifluoroacetic acid.

With the two dipyrrylmethane moieties **1** and **2** in hand, the condensation reaction was carried in a mixture of acetic acid, acetanhydride and trifluoromethyl sulfonic acid (Scheme 1). The desired porphyrin **3** was indeed obtained in excellent 76% yield. The target protoporphyrin IX (**16**) and its disodium salt **17** were obtained as shown in Scheme 4 via reduction of the two acetyl groups with $NaBH_4$ to give the hematoporphyrin ester **15**,

Scheme 3



Scheme 4



followed by elimination of the intermediately formed dibenzoates in good to excellent yields. While **16** was obtained as violet-black crystals of high purity, the disodium salt was isolated as an amorphous powder.

As can be seen from the detailed Experimental Section, the synthesis of **17** was first carried out on a 6 g scale. While most of intermediates could be isolated via distillation or crystallization, some compounds had to be purified by column chromatography. In an intensive process development, all of these chromatographic steps could be circumvented by relatively small variations of the experimental conditions. The improved procedure was then applied to the preparation of protoporphyrin IX derivatives on a 100 g scale with an overall yield in the range of 40% starting from **1** and **2**.

Conclusions

Protoporphyrin IX dimethyl ester **16** and its disodium salt **17** can be prepared from commercially available starting materials via a modified MacDonald procedure. The key step is the condensation reaction of the diiodo dipyrrylmethane **1** with the dialdehyde **2** which directly furnishes the porphyrin **3** without the need of oxidation of a dihydroporphyrin intermediate. Both dipyrrylmethane units can be prepared on multi 100 g scale in excellent purity and satisfactory yield without the need of chromatography.

Experimental Section

General Comments. Reagents and solvents were used as received from common commercial vendors. Methods: Generic RP-HPLC method: Hypersil BS-C C18, 125 mm × 4 mm, 25 °C, solvents with 0.1% TFA, acetonitrile(ACN)/water from 1 to 100% ACN in 10 min, then 2 min 100% ACN. Flow rate 1 mL/min, detection at 220, 410 nm, typical sample concentration 1 mg/mL. NMR Bruker (300 MHz), LCMS: YMC-Pack Pro C18 33 mm × 3 mm, 3 μm, gradient methanol/water (0.04% formic acid), ES ionization.

4,3′-Diacetyl-3,4′-dimethyl-5,5′-diiodo-2,2′-dipyrrylmethane (1). A 2.5 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel and an argon conduit is loaded with solid $NaHCO_3$ (55.6 g, 662.0 mmol), water (900 mL) and ethanol (300 mL). A solution of the compound **11** (54.3 g, 101.9 mmol) in ethanol (300 mL) is added. A solution of iodine (64.7 g, 254.9 mmol) in ethanol (400 mL) is added at ambient temperature to give a brown heterogeneous mixture. A certain amount of foaming and some heating are observed. The conversion is followed by HPLC. The reaction mixture is stirred again at ambient temperature for 5 h. The reaction mixture is diluted with water (0.1 L), and the precipitated product is isolated by filtration. The precipitate is washed with water (3 × 0.1 L), ethanol (2 × 0.1 L), and ether (2 × 0.1 L). After drying of the crystals of product under reduced pressure at 60 °C, **1** (48.1 g, 92%) is recovered in the form of light-red crystals. Purity: 100%.

MS: 509 (M − 1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.06 (s, CH_3); 2.15 (s, CH_3); 2.30 (s, CH_3); 2.38 (s, CH_3); 4.10 (s, CH_2); 11.20 (s, NH); 11.34 (s, NH).

5,5′-Diformyl-4,4′-dimethyl-3,3′-di(2-methoxycarbonylethyl)-2,2′-dipyrrylmethane (2). A 1 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel and an argon conduit is loaded with trifluoroacetic acid (190 mL) and cooled to 0 °C. The compound **14** (20.0 g, 46.0 mmol) is added in small portions in 10 min at 0 °C. The mixture is stirred again at 0 °C for 1 h. The conversion is followed by HPLC. Trimethyl orthoformate (57 mL) is added dropwise in 30 min, while the temperature is maintained at between 0 and 5 °C. The reaction mixture is stirred for a further 1 h at 0 °C and then poured into water (1.7 L). The mixture is stirred vigorously for 10 min. The precipitated crude product is isolated by filtration and washed with water (0.3 1) in the form of an orange powder. The crude product is triturated in ethanol (0.2 L) and ammonia (0.4 L). The mixture is stirred for 30 min at ambient temperature, and the product is isolated by filtration and washed with water (0.3 L) in the form of a dark-yellow powder. The product is refluxed in methanol (0.4 L) for 10 min. The mixture is cooled to ambient temperature, and the product is isolated by filtration and washed with cold methanol (0.1 L). The product is dried under reduced pressure to give **2** (15.30 g, 83%) in the form of a light-yellow powder. Purity: 97%.

MS: 435 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 2 CH_3); 2.52 (t, 2 CH_2); 2.81 (t, 2 CH_2); 3.70 (s, 2 CH_3); 4.05 (s, CH_2); 9.46 (s, 2 CH═O); 10.42 (br s, 2 NH).

Diacetyl-deuteroporphyrin-dimethylester (3). A 2.5 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with acetic acid anhydride (290 mL), acetic acid (1.8 L), and trifluoromethane sulfonic acid (4.95 mL, 56.77 mmol). A substantially homogeneous solution of the compound **2** (12.00 g, 29.82 mmol) and of the compound **1** (14.48 g, 28.40 mmol) in acetic acid (400 mL) is added at ambient temperature in 5 min, which produces a blood-red solution. No exothermic is observed. The mixture is stirred again at ambient temperature for 1 h, with the formation of a certain precipitate. The conversion is followed by HPLC. A solution of NaOAc (9.4 g) in acetic acid (100 mL) is added to give a dark-brown solution. After l0 min, the volatile products are removed under reduced pressure and dried under reduced pressure for 1.5 h at 50 °C. The dark residue is taken up in dichloromethane (300 mL), and water (500 mL) without vigorous mixing. The organic layer is separated, and the aqueous phase is extracted with dichloromethane (0.3 L). The combined organic phase is dried (Na_2S0_4), filtered, and concentrated to give a black crystalline product (31.3 g). The mixture is dissolved in dichloromethane and applied to a column of silica gel C 1 kg) covered with dichloromethane/acetone (95/5). The product is eluted with a gradient of 95/5 to 90/10. The fractions contained in the product are combined and completely concentrated under reduced pressure. The compound **3** (9.97 g, 55%) is recovered in the form of violet-black crystals. Purity: 97% (HPLC, 410 nm).

MS: 623 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ −3.8 (s, NH); −3.6 (s, NH); 3.15 (2 t, 2 CH_2); 3.17 (s, CH_3); 3.25 (s, CH_3); 3.39 (s, CH_3); 3.50 (s, CH_3); 3.60 (s, CH_3); 3.64 (s, CH_3); 3.66 (s, CH_3); 3.71 (s, CH_3); 4.22 (2 t, 2 CH_2); 9.50 (s, CH); 9.59 (s, CH); 10.43 (s, CH); 10.46 (s, CH).

Pent-3-yne-2-one (5). A 4.5 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with aluminum trichloride (297 g, 2.23 mol) and dichloromethane (2.3 L) and cooled to 0 °C. A solution of trimethylsilylpropyne **4** (250 g, 2.23 mol) and acetyl chloride (0.16 L, 2.23 mol) in dichloromethane (0.4 L) was added to the light-yellow suspension in 1.5 h, the temperature being maintained at between 0 and 5 °C. The brown solution with a certain amount of precipitated salt is heated to ambient temperature. The resulting reddish-brown solution is poured into ice/water (2 L). The layers are separated, and the aqueous phase is extracted with dichloromethane (0.5 L). The combined organic phase is washed with water (0.5 L), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a greenish-black liquid (558 g). The product is distilled at 180 mbar to give a fraction (∼160 g) that boils between 64 and 70 °C. This product is again distilled at 210 mbar to give the compound **5** (90.30 g, 49%) in the form of a colorless liquid that has a boiling point between 81 and 85 °C. Purity: 97%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.02 (s, CH_3); 2.31 (s, CH_3).

4-Acetyl-2-benzyloxycarbonyl-4-methyl-1H-pyrrole (6). A l-L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded successively with isocyanoacetic acid benzyl ester (commercial product, Priaxon, Munich) (101.90 g, 0.58 mmol), dioxane (0.5 I), and the compound **5** (52.53 g, 0.64 mol). When methyldiphenylphosphine (38.4 g, 0.19 mol) is added, the reaction becomes highly exothermic. The reaction mixture becomes dark and is heated at 100 °C for 1 h. The conversion is followed by HPLC. The volatile products are removed under reduced pressure. The crude brown oil (209 g) is purified by chromatography on silica (2.1 kg), elution being carried out with a toluene/ethyl acetate (8:1) mixture. The fractions containing the pure product are combined, and the volatile products are removed under reduced pressure to give **6** (73.49 g, 49%) in the form of a light-brown, syrupy oil.

^{1}H NMR (300 MHz, $CDCI_3$): δ 2.13 (s, CH_3), 2.54 (s, CH_3), 5.32 (8 s, CH_2), 6.68 (d, CH), 7.38 (m, 5H), 9.20 (br s, NH).

4-Acetyl-2-benzyloxycarbonyl-3,5-dimethyl-1H-pyrrole (8). A 2.5 L round-bottomed flask equipped with a reflux condenser/distillation head, a thermometer, a dropping funnel, and an argon conduit is loaded with a solution of ethyl 4-acetyl-3,5-dimethy-Ipyrrole-2-carboxylate (commercial product, Alpha Aesar, Karlsruhe, Germany, product No. A 17365) (146.00 g, 0.70 mol) in benzyl alcohol (1 L) and heated to 120 °C, which results in the azeotropic removal of minor amounts of water. The mixture is then heated to 190 °C. The dropping funnel is loaded with a separately prepared solution of sodium (2 g) in benzyl alcohol (20 mL). This solution is added in 5 mL portions, which results in a vigorous reflux of the reaction mixture. The resulting methanol and ethanol are removed semicontinuously by distillation. The conversion is followed by HPLC. The reaction mixture is cooled to 150 °C and then transferred into a mixture of methanol (0.96 L), water (0.66 L), and acetic acid (12 mL). The mixture is cooled to −10 °C and again stirred at this temperature for 1.5 h. The precipitated product is isolated by

filtration. The product is dried under reduced pressure to give **8** (124.40 g, 65%) in the form of an off-white solid. Purity: 100%.

^{1}H NMR (300 MHz,$CDCl_3$): δ 2.34 (s, CH_3); 2.40 (s, CH_3); 2.52 (s, CH_3); 5.23 (s, CH_2); 7.85 (m, 5H); 9.55 (br s, NH).

5-Acetoxymetyl-4-acetyl-2-benzyloxycarbonyl-3-methyl-1H-pyrrole (9). A 2.5 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with compound **8** (66.84 g, 0.25 mol), acetic acid (1.25 L), and sodium acetate (73.90 g, 1.51 mol). In order to obtain a clear solution, the mixture is heated to 35 °C and then cooled to ambient temperature. Sulfuryl chloride (32.4 mL, 0.40 mol) is added in 2 h, while the reaction is carefully controlled toward the end of the addition, in order to minimize byproduct formation due to overreaction. Additional amounts of sodium acetate (50.0 g) are added at ambient temperature, and the mixture is again stirred at ambient temperature overnight. Water (500 mL) is added to give a clear solution. After the addition of a 9:1 water/methanol mixture (4.5 L), the reaction mixture is again stirred at ambient temperature for 1 h with precipitation of the product. The product is isolated by filtration and dissolved by refluxing in ethyl acetate (220 m). The two-phase mixture is removed from the oil bath, and methanol (200 mL) is added with stirring. After further stirring for 1 h at ambient temperature, the product begins to crystallize. Additional amounts of methanol (500 mL) are added, and the mixture is stirred and cooled to −10 °C. The product is isolated by filtration. The product is dried under reduced pressure to give **9** (37.14 g, 45%) in the form of a white solid. Purity: 92%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.07 (s, CH_3); 2.41 (s, CH_3); 2.53 (s, CH_3); 5.27 (s, CH_2); 5.31 (s, CH_2); 7.32 (m, 5H); 9.40 (br s, NH).

4,3′-Diacetyl-5,5′-dibenzyloxycarbonyl-3,4′-dimethyl-2,2′-dipyrrylmethane (10). A 2.5 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with the compound **6** (50.0 g, 194.4 mmol), the compound **9** (51.3 g, 155.6 mmol), and dichloroethane (1.1 L). The mixture is heated to 40 °C to give an orange-red solution. HBF_4 etherate (2.35 mL (54%), 9.3 mmol) is added, and the mixture is heated rapidly to 90 °C. The conversion is followed by HPLC. After 1 h, the mixture is cooled rapidly to ambient temperature and poured into a saturated bicarbonate solution (0.5 L). The layers are separated and the aqueous phase is extracted with dichloroethane (0.5 L). The combined organic extracts are dried (Na_2SO_4), filtered, stirred with Norrit C (2 g), filtered, and completely concentrated under reduced pressure to give a sticky, brown syrup (96.5 g). The crude product is dissolved in methanol (0.3 L), concentrated under reduced pressure, and again dissolved in methanol (150 mL). Germination crystals are added, and the mixture is left to stand for 15 h at ambient temperature while the product crystallizes. The supernatant is removed, and the crystals (fraction KI, 34.3 g) are washed with methanol. The combined methanol fractions are completely concentrated under reduced pressure and chromatographed on silica (420 g), elution being carried out with hexane/ethyl acetate (2:1). The fractions containing the product are combined and concentrated under reduced pressure. The product is recrystallized as above in methanol to give a fraction K2 (16.7 g). The supernatant is again chromatographed on silica (400 g), elution being carried out with hexane/ethyl acetate (2:1). The fractions containing the product are combined and concentrated under reduced pressure. The product is recrystallized as above from methanol to give a fraction K3 (3.7 g). The product fractions (KI−K3) are combined, dissolved in toluene, and completely concentrated under reduced pressure. After drying under reduced pressure at 50 °C for 1 h, **10** (54.7 g, 68%) is recovered in the form of off-white crystals. Purity: 93%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.09 (s, CH_3); 2.49 (s, CH_3); 2.51 (s, CH_3); 2.58 (s, CH_3); 4.04 (s, CH_2); 5.27 (s, CH_2); 5.29 (s, CH_2); 7.35 (m, 10H); 10.5 (br s, 2 NH).

4,3′-Diacetyl-5,5′-dicarboxy-3,4′-dimethyl-2,2′-dipyrrylmethane (11). A low-pressure hydrogenation apparatus is loaded with the compound **10** (54.3 g, 103.1 mmol), tetrahydrofuran (700 mL), triethylamine (20.8 g, 206.2 mmol), and 10% Pd/C catalyst (2.75 g). The hydrogenation is carried out at ambient temperature under a hydrogen pressure atmosphere in 3 h. The catalyst is removed by filtration. The filtrate is concentrated under reduced pressure. After drying under reduced pressure at 45 °C for 0.5 h, **11** (54.7 g, quantitative) is recovered in the foam of an off-white foam as monotriethylamine salt containing residual amounts of toluene and THF. Purity: 96%.

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.10 (t, CH_3); 1.97 (s, CH_3); 2.38 (s, CH_3); 2.52 (s, 2 CH_3); 2.85 (q, CH_2); 4.14 (s, CH_2); 11.36 (br s, NH); 11.70 (br s, NH).

*5,5′-Dibenzyloxycarbonyl-3,3′-di(2-methoxycarbonylethyl),4,4′-dimethyl-2,2′-dipyrrylmethane (**13**).* A 4.5 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with the compound **12** (52.0 g, 165.0 mmol) and TBME (1.5 L).A freshly prepared solution of bromine (11.0 mL, 214.0 mmol) in TBME (0.5 L) is added dropwise in 20 min at ambient temperature, so as to produce an orange-brown solution. The conversion is followed by HPLC. If necessary, additional amounts of bromine are added. The mixture is again stirred at ambient temperature. The volatile products are removed under reduced pressure, and the grayish-brown residue is dissolved in methanol (364 mL), and 0.4 mL of conc. HCl is added. The solution is heated at ∼50 °C until complete conversion is obtained (determined by HPLC after approximately 11 h). The dark reaction mixture is concentrated under reduced pressure until the product begins to crystallize. The precipitated product is isolated by filtration and washed with methanol (0.2 L). The crude product is recrystallized by suspending in diethyl ether (0.6 L) and refluxing, while heptane (1.8 L) is added, and the heating is continued so as to maintain the mixture at reflux for a further 15 min. The mixture is cooled to ambient temperature, and the product is isolated by filtration. The product is dried to give **13** (31.70 g, 63%) in the form of a light-grey powder. Purity: 98%.

MS: 615 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 2 CH_3); 2.43 (t, 2 CH_3); 2.68 (t, 2 CH_2); 3.50 (s, 2 CH_3); 3.89 (s, CH_2); 5.17 (s, 2 CH_2); 7,20 (m 10H); 9.00 (br s, 2 NH).

5,5′-Dicarboxy-3,3′-di(2-methoxycarbonylethyl)-4,4′-dimethyl-2,2′ dipyrrylmethane (14). A low-pressure hydrogenation apparatus is loaded with the compound **13** (30.7 g, 49.9 mmol), THF (400 mL), and 10% Pd/C catalyst (1.5 g, A027). The hydrogenation is carried out at ambient temperature under a hydrogen pressure atmosphere in 3 h. Ammonia (0.1 L of 2 N solution) is added to the reaction mixture, and the catalyst is removed by filtration. The filtrate is adjusted to pH 7 by adding acetic acid (60 mL). The solvent is removed under reduced pressure. The precipitated product is isolated by filtration to give, after drying, **14** (21.7 g, quantitative) in the form of a white powder. Purity: 98%.

MS: 434; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.18 (s, 2 CH_3); 2.20 (t, 2 CH_2); 2.59 (t, 2 CH_2); 3.60 (s, 2 CH_3); 3.82 (s, CH_2); 11.0 (s, COOH); 11.95 (br s, 2 NH).

Hematoporphyrin-dimethylester (15). A 1-L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with the compound **3** (9.86 g, 15.84 mmol), dichloromethane (500 mL), and methanol (24 mL). $NaBH_4$ (3.00 g, 79.32 mmol) is added in portions to the reddish-brown mixture. A certain foaming is observed. The reaction is closely followed by HPLC. After 80 min, the mixture is poured into a mixture of water (500 mL) and 4 N HCI (80 mL). Gas is seen to be given off. The mixture is neutralized by adding solid $NaHCO_3$. The layers are separated, and the aqueous phase is extracted with dichloromethane (2 × 300 mL). The combined organic extracts are dried (Na_2SO_4), filtered, and concentrated under reduced pressure. After drying under reduced pressure at 50 °C for 0.5 h, **15** in the form of a mixture of two stereoisomers (9.58 g, 96%) is recovered in the form of violet-black crystals. Purity: 98% (HPLC, 410 nm); 2 isomers (1:1).

MS: 625 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.92 (m, 6H, 2 CH_3), 3.16 (m, 4H, 2 CH_2), 3.28, 3.30, 3.33, and 3.35 (4 s, 6H, 2 CH_3), 3.43 (s, 6H, 2 CH_3), 3.66 (s, 6H, 2 CH_3), 4.20 (m, 4H, 2 CH_2), 6.05 (m, 2H, 2 CH), 9.73, 9.74, 9.75, 9.76, 10.00, 10.02, 10.08, and 10.10 (8 s, total 4H, 4 CH).

Protoporphyrin IX dimethylester (16). A 1 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with the compound **15** (9.48 g, 15.12 mmol) and DMF (400 mL), and the mixture is degassed with argon. Benzoyl chloride (45.0 mL, 387.9 mmol) is added, and the mixture is rapidly heated to 100 °C. The mixture is stirred again at 100 °C for 1 h. The conversion is followed by HPLC. The reaction mixture is cooled rapidly, and the volatile products are removed under reduced pressure. The residue is dissolved in dichloromethane (0.3 L) and stirred vigorously with a water/methanol (0.3 L) mixture. The layers are separated and the aqueous phase is extracted with dichloromethane (2 × 0.2 L). The combined organic extracts are washed with bicarbonate (0.3 L), dried (Na_2SO_4) and filtered. The filtrate is treated with silica (20 g) and filtered. Methanol (50 mL) is added and the mixture is then concentrated under reduced pressure, while crystallization takes place toward the end to give a violet-black product (20 g). The product is triturated with methanol at 50 °C for 0.5 h. After cooling to ambient temperature, chloroform (2 mL) is added, and the product is isolated by filtration. After drying under reduced pressure at 50 °C for 15 h, **16** (6.34 g, 77%) is recovered in the form of shiny violet-black crystals. Purity: 97% (HPLC, 410 nm).

MS: 591 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.23 (t, 2 CH_2); 3.52 (s, CH_3); 3.54 (s, CH_3); 3.58 (s, CH_3); 3.64 (s, CH_3); 3.65 (s, CH_3); 3.66 (s, CH_3); 4.32 (t, 2 CH_2); 6.11−6,34 (m, 4 H, 2 CH_2═C); 8.10−8,23 (m, 2 H, 2 CH═C); 9.85, 9.86, 9.97, and 9.99 (4 s, 4 CH).

Protoporphyrin IX Disodium Salt (17). A 2.5 L round-bottomed flask equipped with a reflux condenser, a thermometer, a dropping funnel, and an argon conduit is loaded with the compound **16** (6.30 g, 10.67 mmol) and dichloromethane (200 mL). The product is dissolved by heating to 40 °C. At 40 °C, methanol (400 mL) followed by 4 N NaOH (200 mL) is successively added. The formation of a precipitate is observed. The mixture is refluxed. The conversion is followed by HPLC. The organic volatile products are removed under reduced pressure. The suspension is filtered through a glass fiber filter. The product is washed with water (3 × 0.1 L), methanol (3 × 30 mL), and diethyl ether (2 × 3 0 mL).After drying under reduced pressure at 70 °C for 2 h, then at 40 °C for 15 h, **17** (6.06 g, 94%) is recovered in the form of a violet-black solid product. Purity: 97% (HPLC, 410 nm).

MS: 563 (M + 1), diacid; ^{1}H NMR (300 MHz, TFA-d_1): δ 3.45 (2t, 2 CH_2); 3.82 (s, CH_3); 3.85 (s, CH_3); 3.88 (s, CH_3); 3.91 (s, CH_3); 4.73 (2t, 2 CH_2); 6.43−6.70 (m, 4 H, 2 CH_2═C); 8.28−8.40 (m, 2 H, 2 CH═C); 11.08, 11.11, 11.15, and 11.27 (4 s, 4 CH).

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