Convenient Preparation of Pheophytin *b* from Plant Extract through the C7-Reduced Intermediate

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A new method for the large scale preparation of Pheophytin (=Pheo) *b* is presented. The difficulty in chromatographic isolation of Pheo *a* and *b*, tetrapyrrole pigments, from the large amount of plant extract was overcome by an increase in polarity of the pigment given by a selective modification of Pheo *b*. Treatment of plant extract containing both Pheo *a* and *b*, carotenoids, lipids, etc. with a mild reductant *t*-$BuNH_2BH_3$ induced selective reduction of the C7-formyl group of Pheo *b*, but left unaffected other carbonyl moieties of the molecules. The resulting compound 7-hydroxymethyl-Pheo *b* was readily separated from intact Pheo *a* by silica gel column chromatography. Pure Pheo *b* was obtained by oxidation of the 7-hydroxymethyl-Pheo *b* with pyridinium chlorochromate. One of the reasons why the present method is superior to the previous ones is that Pheo *a* was obtained simultaneously as an intact form. Another is the utility of the intermediate compound, 7-hydroxymethyl-Pheo *b*: as the starting material for syntheses of various compounds as well as a model of ingredients in the photosynthetic systems, such as a light-harvesting apparatus of green photosynthetic bacteria.

Pheophytin (=Pheo) *b*, demetallated compound (M=H_2) of chlorophyll (=Chl) *b*, has been utilized for the starting material for syntheses of various chlorin compounds due to the presence of a reactive formyl group at the C7 position (Fig. 1).[1,2] Recently increasing attention has been paid to the pigment in relation to photoexcited pheophorbide *b* (Pheid *b*, R_{17}=H) as an artificial restriction endonuclease[3] or to 7-deformyl-7-hydroxymethyl-Chl *b* (C7-reduced compound of Chl *b*) as an intermediate of the in vivo Chl *a* and *b* metabolic cycle.[4]

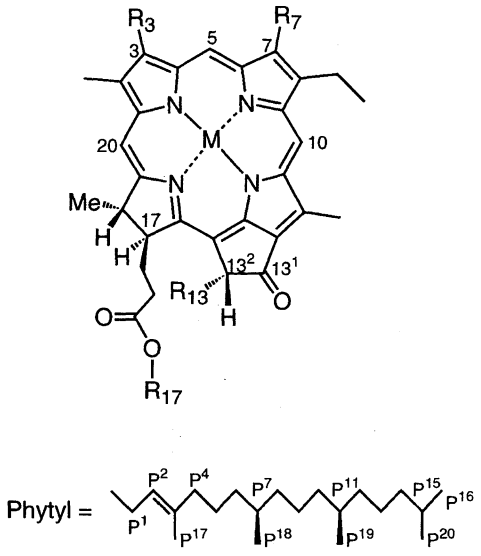


Fig. 1. Molecular structure of metallochlorins. Chl *a*: M=Mg, R_3=CH=CH_2, R_7=Me, R_{13}=COOMe, R_{17}=phytyl; Chl *b*: M=Mg, R_3=CH=CH_2, R_7=CHO, R_{13}=COOMe, R_{17}=phytyl; Chlorosomal Chl (one of bacteriochlorophylls *d*, see Ref. 24): M=Mg, R_3=CH(Me)OH, R_7=Me, R_{13}=H, R_{17}=farresyl; Zn-3^1-OH-Chl: M=Zn, R_3=CH_2OH, R_7=Me, R_{13}=H, R_{17}=Me; Zn-7^1-OH-Chl (**6**): M=Zn, R_3=CH=CH_2, R_7=CH_2OH, R_{13}=H, R_{17}=phytyl.

Pheo *a* can be readily obtained from several organisms which have Chl *a* as the only chlorophyllous pigment.[5,6] On the contrary, because Chl *b* is embedded only in LHC I and II of higher plants as a minor pigment, efficient separation of Pheo *b* (or Chl *b*) from Pheo *a* (or Chl *a*) is indispensable for the preparation of Pheo *b* from the pigment mixture containing chlorophylls, carotenoids, lipids, etc. One can employ chromatographic technique or utilize chemical reactions to prepare Pheo *b*. The former way is hampered by difficulties in large scale separation of Pheo *a*/*b* (or Chl *a*/*b*) by usual silica-gel column chromatography because of the similar polarities. The best way for the chromatographic separation of Chl *a*/*b* is to utilize a quick silica gel-column chromatography followed by a silica normal-phase HPLC, which afforded quite pure pigments (> 99.9%).[7,8] However, the chromatographic method consumes time and large amounts of solvents, and is appropriate in the case that relatively small amounts (less than 10 mg) of quite pure pigments are required. The other methods for the preparation of Pheo *b* by use of molecular modification allow one to deal with larger amount of pigments. There were two reports on utilizing selective hydrolysis of the C17-phytyl ester of Pheo *a*[9] or selective formation of a Schiff base of Pheo *b* by Girard's reagent T.[10] However, these techniques gave Pheid *a*/Pheo *b* or Pheo *a*/Pheid *b*; either Pheo *a* or *b* had to be transformed to the denatured form (R_{17}=H).

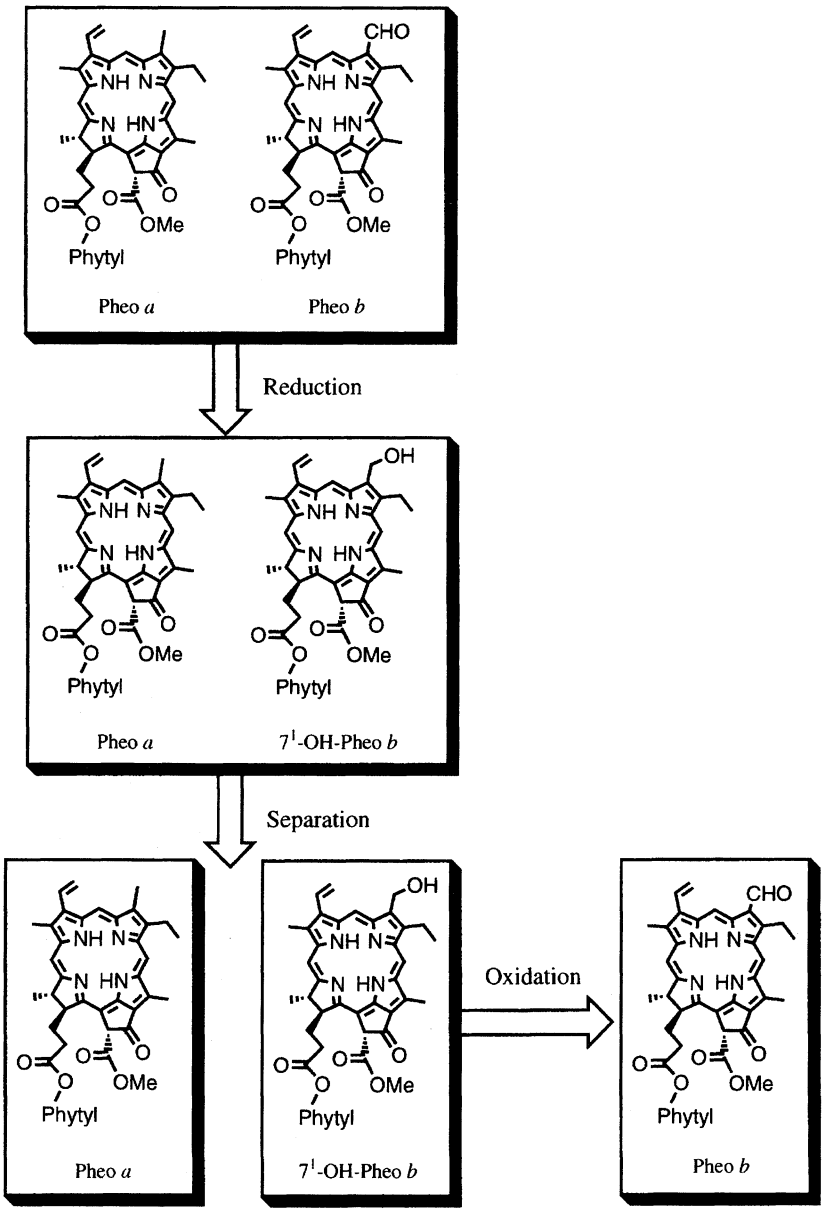
Here we report a novel, convenient method for the large scale preparation of Pheo *b* with simultaneous preparation of

intact Pheo *a*. The C7-formyl group of Pheo *b* is selectively reduced to afford 7-hydroxymethyl-Pheo *b* (7^1-OH-Pheo *b*) in the mixture of Pheo *a*/*b* given from extracts of green plants by organic solvents, and the reduced compound is readily separated from Pheo *a* and other pigments by silica gel column chromatography on the large scale (Scheme 1). The isolated 7-hydroxymethyl compound is easily oxidized to give Pheo *b*. The utility of the intermediate compound, 7^1-OH-Pheo *b*, is also discussed.

Results and Discussion

Preparation of Pheo *b* and pyroPheo *b*. Large scale preparation of Pheo *b* is largely influenced by the efficiency of the separation from coexisting Pheo *a*. The previous synthetic methods facilitated the separation by a difference in solubility of Pheid *a*/Pheo *b* in water[9] or by a difference in interaction of Pheo *a* and the ionic semicarbazone of Pheo *b* with silica-gel.[10] We utilized the principle of selective reduction of C7-formyl group of Pheo *b*; the reduction product was readily separated from Pheo *a* by silica-gel column chromatography because of an increase in polarity (Scheme 1). The reduction of the formyl moiety of Pheo *b* had at first been performed with $NaBH_4$[4,11—14] which can cause the additional undesired reduction of the $C13^1$ keto carbonyl moiety, and was improved later to use a milder reagent of $NaBH_3CN$.[1,2,15] We employed a much milder reductant, *t*-$BuNH_2BH_3$, which selectively reduced the formyl group of Pheo *b* and left other carbonyl moieties intact even during a prolonged reaction.

Photosynthetic pigments were extracted from spinach leaf tissues (wet weight, ca. 220 g) by light petroleum ether/methanol (1/1—2). After pheophytinization (demetallation of central magnesium) by treatment of acid, the extract reacted



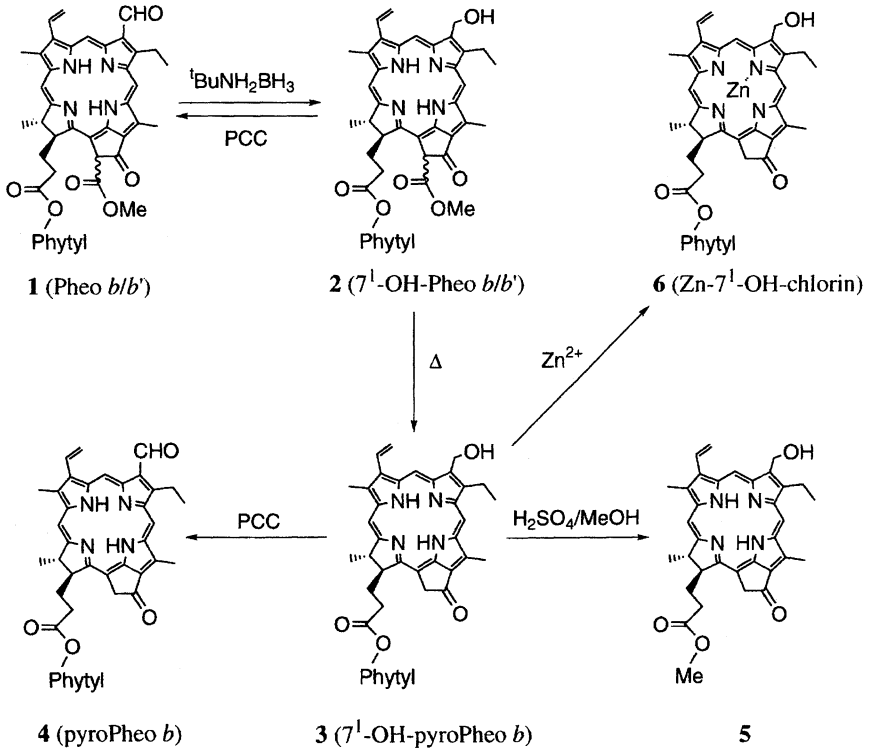
Scheme 1. Strategy for preparation of Pheo *b*.

with *t*-$BuNH_2BH_3$ (ca. 30 mg) in dichloromethane at room temperature. A 438-nm absorption peak of the extract, originating from Pheo *b*, was decreased as Pheo *b* was reduced. Formation of the desired C7-reduced compound **2** was also confirmed by detection of the 7^1-methylene proton resonance (δ =5.75 ppm, in $CDCl_3$) with concomitant disappearance of the formyl proton signal (δ = 11.0 ppm). After 10-h stirring, the reaction mixture was chromatographed on silica-gel. The first colored elution with CH_2Cl_2 gave carotenenoids (orange band). Pheo *a* (ca. 310 μmol, 270 mg)[16] was eluted as the next black band (CH_2Cl_2/diethyl ether=100/1—2). Finally, a deep brown band containing compound **2** (ca. 61 μmol, 54 mg) was eluted with CH_2Cl_2/diethyl ether=100/4—5. Compound **2** was easily oxidized with pyridinium chlorochromate (PCC) for 1-h reaction at room temperature to afford **1** in 70% yield (see Scheme 2), which was confirmed by an appearance of the formyl proton resonance with simultaneous loss of the 7^1-methylene NMR signal (vida supra).

Pheo *b* and 7^1-OH-Pheo *b* have the diastereomers, Pheo *b'* and 7^1-OH-Pheo *b'*, respectively; the latter two 'prime-type' isomers have *S*-configuration at $C13^2$ position (Fig. 1). The *R*/*S*-ratio of **2** was estimated to be ca. 76/24 by areas of *meso*-proton (5-, 10-, and 20-H) resonance peaks. Pheo *b* (**1**) obtained by the oxidation of **2** also had the *S* isomer (ca. 12%). The produced Pheo *b* was in equilibrium with Pheo *b'*, as judged from the previously reported Pheo *b*/*b'* molar ratio at equilibrium (87/13) and quite rapid epimerization (rate constant: 150-fold larger than Chl *a*).[17] Major Pheo *b* (ca. 88%; $C13^2$-*R*-epimer) could be readily purified by reversed-phase HPLC (retention time: Pheo *b*<Pheo *b'*). By pyrolysis in 2,4,6-collidine,[5] compound **2** was readily converted into 7^1-OH-pyroPheo *b* (**3**) which lacked the $C13^2$-methoxycarbonyl moiety with the same π-conjugated system (66% yield; Scheme 2). The oxidation of **3** with PCC yield pyroPheo *b* (**4**) in 45% yield. Transesterification of compound **3** to methyl 7-deformyl-7-hydroxymethyl-pyroPheid *b* (**5**) was also performed by 1-h treatment with methanol containing 10% (vol/vol) of concentrated sulfuric acid (84% yield).

The present separation method is superior to previous ones because the phytyl ester groups of both Pheo *a* and *b* are not affected during the procedures. Hynninen et al.[9] reported that contact of the Pheo *a*/*b* mixture (in diethyl ether) with 30% hydrochloric acid gave Pheid *a* and Pheo *b* based on the difference in hydrolysis rate (Pheo *a* > Pheo *b*), and the phorbins could be separated in a separatory funnel (the upper organic phase with Pheo *b*, the lower aqueous phase with Pheid *a*). Kenner et al.[10] described how the treatment of Girard's reagent T (the ionic semicarbazide) with Pheo *a*/*b* mixture resulted in the selective formation of Schiff base (semicarbazone) with the C7-formyl moiety of Pheo *b*, whereas Pheo *a* was unaffected, and then the ionic Schiff base produced was separated from Pheo *a* by the silica gel chromatography. However, cleavage of the C=N bond of the Schiff base with concentrated sulfuric acid also caused hydrolysis of the phytyl ester. Thus, either Pheo *a* or *b* had to be hydrolyzed during these two preparations. Additionally, all the separated Pheo/Pheids were $C13^2$-epimeric mixtures, *a*/*a'* and *b*/*b'*. Our method readily yielded Pheo *b*[16] and further afforded intact Pheo *a*[16] simultaneously in a relatively large amount (10 mg—1 g),[16] which is an advantage over the previous methods.

Synthesis of Zn 7^1-OH-chlorin as a Model Compound



Scheme 2. Syntheses of 7-hydroxymethyl-Pheo *b* derivatives.

of Chlorosomal Chlorophylls. Another advantage of the present method is that formed 7^1-OH compounds **2** and **3** are useful as the starting material for syntheses of various compounds as well as a model of ingredients in the photosynthetic systems. Magnesium complex of 7^1-OH-Pheo *b* was found to be an intermediate in the in vivo transformation between Chl *a* and *b*.[4] The hydroxymethyl groups of **3** was esterified to obtain various chlorin compounds.[1,2] We here present another significance of 7^1-hydroxylchlorin derivatives in the study of the structure and function of chlorosome, an extramembranous light-harvesting apparatus of green photosynthetic bacteria, which currently attracts numerous researchers.[5,18] Chlorosomal Chls all possess a 1-hydroxyethyl group at C3 position (Fig. 1) and tend to associate together with the simultaneous coordination/hydrogen-bonding of $C3^1$–OH to Mg and $C3^1$–OH to $C13^1$=O.[5] The analogue 7^1-OH-type chlorin was also expected to form self-aggregates.

The chlorosomal Chl analogue, zine 7-deformyl-7-hydroxymethyl-pyroChl *b* (**6**), was synthesized by zinc-metallation of compound **3** with $Zn(OAc)_2$, according to the reported procedures (Scheme 2).[5] Synthetic Zn-7^1-OH-chlorin **6** readily self-aggregated in nonpolar organic solvents to form oligomers in a manner similar to that used with synthetic Zn-3^1-OH-chlorins and natural occurring chlorosomal Chls.[5,19] Elucidation of the supramolecular structure of the self-aggregate is now in progress; results will be reported elsewhere.

Experimental

Materials and Apparatus: Reagents and solvents were purchased from Nacalai Tesque (Kyoto, Japan).

Chlorophyllous pigments were isolated either by flash column chromatography (FCC) with silica-gel (Merck, Kieselgel 60, 9385) or by reversed-phase HPLC (ODS column: GL-OP100 6.0 mmϕ×150 mm (Hitachi Chemical Co., Ltd.), room temperature; eluent: methanol; flow rate: 1.0 ml min^{-1}; detector: Shimadzu absorbance detector Model SPD-10A, 427 nm).

All the compounds were characterized by ^{1}H NMR, mass, and visible absorption spectra. Proton NMR spectra were recorded on a 300 MHz NMR spectrometer (Bruker AC-300) in $CDCl_3$ solutions (20 °C). Resonance peaks were assigned on the basis of NOESY and COSY measurements and the literature data.[5,20,21] Mass spectra were obtained by use of a fast atom bombardment mass spectrometer JEOL HX-110 (FAB matrix, 3-nitrobenzyl alcohol). Visible absorption spectra were recorded on a Hitachi spectrophotometer U-3500.

Syntheses: 7-Deformyl-7-hydroxymethyl-pheophytin *b*/*b*′ (=7^1-OH-Pheo *b*/*b*′, 2): Photosynthetic pigments were extracted from spinach leaf tissues (wet weight, ca. 220 g) with light petroleum/methanol (1/1—2). Successively, the extracted solution, which contained water, was contacted with diluted hydrochloric acid (0.4%) to demetallate the chlorophyllous pigments. The upper black organic phase was separated and concentrated in vacuo. The residue was dissolved in CH_2Cl_2, and the solution was washed with aqueous saturated $NaHCO_3$ solution and brine, followed by drying over Na_2SO_4.

After evaporation, the plant extract (roughly 3 g) was dissolved into CH_2Cl_2. To this solution was added *t*-butylamine–borane complex (1/1) (30 mg) at 0 °C and stirred for 10 h at room temperature. The progress of the reduction was monitored by a decrease of the 438-nm absorption of Pheo *b*. The reaction mixture was washed with aq 3% HCl, aq saturated $NaHCO_3$ solution and brine, then dried over Na_2SO_4. A brown solution of compound **2** was isolated by FCC with CH_2Cl_2/diethyl ether (100/4—5) as the third fraction (the first, carotenoids (100/0); the second, ca. 310 μmol (270 mg) of Pheo *a* (100/1—2)). Compound **2** was reprecipitated from CH_2Cl_2/hexane (dark solid, 54 mg, 61 μmol; a $C13^2$-epimeric mixture of R/S=76/24). Visible absorption (CH_2Cl_2): λ_{max} 662 (rel intensity, 0.36), 606 (0.07), 541 (0.07). 510 (0.10), 419 nm (1.00). ^{1}H NMR ($CDCl_3$, only the *R*-isomer's data is presented) δ=9.62, 9.60, 8.58 (3s, CH-10+5+20), 8.00 (dd, J=12, 18 Hz, $CH\text{-}3^1$), 6.31 (dd, J=1, 18 Hz, $CH\text{-}3^2$-*cis*), 6.26 (s, $CH\text{-}13^2$), 6.19 (dd, J=1, 12 Hz, $CH\text{-}3^2$-*trans*), 5.75 (s, $CH_2\text{-}7^1$), 5.14 (t, J=7 Hz, $CH\text{-}P^2$), 4.40—4.54 (m, CH-18+$CH_2\text{-}P^1$), 4.21 (dt, J=2, 8 Hz, CH-17), 3.89, 3.69, 3.40 (3s, $CH_3\text{-}13^5$+12^1+2^1), 3.80 (q, J=8 Hz, $CH_2\text{-}8^1$), 2.40—2.72, 2.10—2.40 (2m, $CH_2\text{-}17^1$+17^2), 1.88 (t, J=7 Hz, $CH_2\text{-}P^4$), 1.81 (d, J=7 Hz, CH_3-18), 1.76 (t, J=8 Hz, $CH_3\text{-}8^2$), 0.65—1.70 (m, 34 H on P^5–P^{20}), −1.65 (s, NH^{22}). MS (FAB) *m*/*z* 887.6 (MH^+).

Pheophytin *b*/*b*′ (=Pheo *b*/*b*′, 1): Pyridinium chlorochromate (PCC, 2.4 mg) was dissolved in CH_2Cl_2 (10 ml), and compound **2** (4.9 mg, 5.5 μmol) was added into the solution. After stirring for 1 h at room temperature, the pigment was extracted with diethyl ether. The solution was washed with brine, and dried over Na_2SO_4. On FCC, **1** was eluted with CH_2Cl_2/diethyl ether=100/2 as a $C13^2$-epimeric mixture of R/S=88/12 (3.9 μmol, 70% yield). NMR and MS data were consistent with those in the literature.[20,21,23] Visible (CH_2Cl_2) λ_{max} 655 (0.20), 600 (0.06), 528 (0.08), 439 (1.00), 417 nm (0.42). ^{1}H NMR ($CDCl_3$, the *R*-isomer's data is presented) δ=11.0, (s, $CH\text{-}7^1$), 10.2, 9.43, 8.53 (3s, CH-10, 5, 20), 7.95 (dd, J=12, 18 Hz, $CH\text{-}3^1$), 6.34 (dd, J=1, 18 Hz, $CH\text{-}3^2$-*cis*), 6.25 (s, $CH\text{-}13^2$), 6.21 (dd, J=1, 12 Hz, $CH\text{-}3^2$-*trans*), 5.16 (t, J=7 Hz, $CH\text{-}P^2$), 4.25—4.60 (m, CH-18+$CH_2\text{-}P^1$), 4.20 (dt, J=3, 9 Hz, CH-17), 3.93, 3.63, 3.37 (3s, $CH_3\text{-}13^5$, 12^1, 2^1), 3.85 (q, J=8 Hz, $CH_2\text{-}8^1$), 2.45—2.85, 2.15—2.45 (2m, $CH_2\text{-}17^1$+17^2), 1.89 (t, J=7 Hz, $CH_2\text{-}P^4$), 1.85 (d, J=7 Hz, CH_3-18), 1.73 (t, J=8 Hz, $CH_3\text{-}8^2$), 0.70—1.70 (m, 34 H on P^5–P^{20}), 0.38, −1.68 (2s, NH). MS (FAB) *m*/*z* 885.7 (MH^+).

7-Deformyl-7-hydroxymethyl-13^2-demethoxycarbonyl-pheophytin *b* (=7^1-OH-pyroPheo *b*, 3): According to literature procedures,[5] reflux of compound **2** in 2,4,6-collidine (2,4,6-trimetylpyridine) followed by the isolation using FCC gave dark green compound **3**. Yield: 66%. Visible (CH_2Cl_2) λ_{max} 662 (0.38), 606 (0.07), 542 (0.06), 512 (0.10), 419 nm (1.00). ^{1}H NMR ($CDCl_3$) δ=9.63, 9.59, 8.58 (3s, CH-10, 5, 20), 8.03 (dd, J=12, 18 Hz, $CH\text{-}3^1$), 6.32 (dd, J=1, 18 Hz, $CH\text{-}3^2$-*cis*), 6.19 (dd, J=1, 12 Hz, $CH\text{-}3^2$-*trans*), 5.77 (s, $CH_2\text{-}7^1$), 5.28, 5.11 (2d, J=20 Hz, $CH_2\text{-}13^2$), 5.23 (t, J=6 Hz, $CH\text{-}P^2$), 4.40—4.60 (m, CH-18+$CH_2\text{-}P^1$), 4.27 (dt, J=3, 8 Hz, CH-17), 3.81 (q, J=8 Hz, $CH_2\text{-}8^1$), 3.67, 3.41 (2s, $CH_3\text{-}12^1$+2^1), 2.15—2.45, 2.45—2.80 (2m, $CH_2\text{-}17^1$+17^2), 1.93 (t, J=8 Hz, $CH_2\text{-}P^4$), 1.81 (d, J=7 Hz, $CH_3\text{-}18^1$), 1.77 (t, J=8 Hz, $CH_3\text{-}8^2$), 0.70—1.70 (m, 34 H on P^5–P^{20}). −1.70 (s, NH^{22}). MS (FAB) *m*/*z* 829.7 (MH^+).

13^2-Demethoxycarbonyl-pheophytin *b* (=pyroPheo *b*, 4): Similarly to the synthesis of **1**, the oxidation of compound **3** gave pyroPheo *b* (**4**) in 45% yield. The structure was confirmed by NMR and MS measurements.[20,21,23] Visible (CH_2Cl_2) λ_{max} 656 (0.19), 601 (0.06), 531 (0.08), 440 (1.00), 419 nm (0.43). ^{1}H NMR ($CDCl_3$) δ=11.2 (s, $CH\text{-}7^1$), 10.3, 9.60, 8.52 (3s, CH-10+5+20), 8.02 (dd, J=12, 18 Hz, $CH\text{-}3^1$), 6.37 (dd, J=1, 17 Hz, $CH\text{-}3^2$-*cis*),

6.22 (dd, J=1, 12 Hz, CH-3^2-*trans*), 5.25, 5.07 (2d, J=20 Hz, CH_2-13^2), 5.22 (t, J=8 Hz, CH-P^2), 4.40—4.60 (m, CH-18+CH_2-P^1), 4.28 (dt, J=2, 8 Hz, CH-17), 4.06 (q, J=8 Hz, CH_2-8^1), 3.65, 3.38 (2s, CH_3-12^1+2^1), 2.15—2.45, 2.45—2.90 (2m, CH_2-17^1+17^2), 1.93 (t, J=7 Hz, CH_2-P^4), 1.82 (d, J=7 Hz, CH_3-18^1), 1.82 (t, J=8 Hz, CH_3-8^2), 0.70—1.75 (m, 34 H on P^5–P^{20}), 0.07, −1.70 (2s, NH). MS (FAB) *m/z* 827.6 (MH^+).

Methyl 7-Deformyl-7-hydroxymethyl-pyropheophorbide *b* (5): Concentrated sulfuric acid (0.5 ml) was added dropwise to an ice-chilled methanol solution (5 ml) of **3** (4.9 mg) and the mixture was stirred for 1 h. The solution was poured into ice water and extracted with CH_2Cl_2. The organic phase was neutralized with aqueous $NaHCO_3$ solution, washing with brine and dried over Na_2SO_4. The residue was purified by FCC (eluted with CH_2Cl_2/diethyl ether = 100/8). Black solid of **5** was obtained by reprecipitation from CH_2Cl_2/hexane (2.8 mg, 84% yield). Visible (CH_2Cl_2) λ_{max} 662 (0.38), 606 (0.07), 541 (0.06). 511 (0.10), 418 nm (1.00). ^{1}H NMR ($CDCl_3$) δ = 9.56, 9.37, 8.53 (3s, CH-5+10+20), 7.98 (dd, J=12, 18 Hz, CH-3^1), 6.29 (dd, J=1, 18 Hz, CH-3^2-*cis*), 6.16 (dd, J=1, 12 Hz, CH-3^2-*trans*), 5.70 (s, CH_2-7^1), 5.19, 5.04 (2d, J=20 Hz, CH_2-13^2), 4.45 (dq, J=2, 7 Hz, CH-18), 4.13—4.38 (dt, J=3, 9 Hz, CH-17), 3.72 (q, J=8 Hz, CH_2-8^1), 3.62, 3.52, 3.39 (3s, CH_3-17^5+12^1+2^1), 2.45—2.75, 2.13—2.38 (2m, CH_2-17^1+17^2), 1.79 (d, J=7 Hz, CH_3-18^1), 1.70 (t, J=8 Hz, CH_3-8^2), 0.25, −1.80 (2s, NH). MS (FAB) *m/z* 564.4 (M^+).

Zinc-7-deformyl-7-hydroxymethyl-13^2-demethoxycarbonyl-pheophytin *b* (Zn-7^1-OH-chlorin, 6): Zinc metallation of compound **3** was performed as described previously.[5] Compound **6** was purified by use of reversed-phase HPLC (retention time = 20 min). The green solid (**6**) was obtained by reprecipitation from CH_2Cl_2/hexane (67% yield). Visible (CH_2Cl_2) 648 (0.56), 601 (0.09), 559 (0.04), 517 (0.04), 427 nm (1.00). ^{1}H NMR (15 vol% CD_3OD/$CDCl_3$) δ = 9.45, 9.28, 8.22 (3s, CH-10, 5, 20), 7.88 (dd, J=11, 18 Hz, CH-3^1), 6.06 (dd, J=1, 18 Hz, CH-3^2-*cis*), 5.90 (dd, J=1, 11 Hz, CH-3^2-*trans*), 5.60 (s, CH_2-7^1), 5.13, 4.93 (2d, J=20 Hz, CH_2-13^2), 5.10 (t, J=7 Hz, CH-P^2), 4.25—4.40 (m, CH-18+CH_2-P^1), 4.11 (dt, J=3+8 Hz, CH-17), 3.72 (q, J=7 Hz, CH_2-8^1), 3.57, 3.25 (2s, CH_3-12^1, 2^1), 2.30—2.60, 2.05—2.30 (2m, CH_2-17^1+17^2), 1.82 (t, J=7 Hz, CH_2-P^4), 1.68 (d, J=7 Hz, CH_3-18^1), 1.65 (t, J=8 Hz, CH_3-8^2), 0.50—1.60 (m, 34 H on P^5–P^{20}). MS (FAB) *m/z* 890.6 (M^+, for ^{64}Zn).

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