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Synthetic Zinc Tetrapyrroles Complexing with Pyridine as a Single Axial Ligand

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Abstract—Zinc chlorins were prepared from chlorophyll-*a*. Visible spectra in benzene showed that synthetic zinc chlorins complexed with pyridine as an axial ligand to form the monopyridine adducts. The equilibrium constants for the complexation were dependent upon the chlorin structure: substitution of electron-withdrawing groups at the peripheral position enhanced the coordinated ability of the central zinc. ^{1}H NMR spectra in benzene-d_6 also indicated that single pyridine coordinated to the central zinc. Comparison of the equilibrium constant in a zinc chlorin with those of the corresponding zinc bacteriochlorin (7,8-dihydrochlorin) and porphyrin (17,18-dedihydrochlorin) led to an increase in the saturation and flexibility of the tetrapyrrole π-plane ligands making the central zinc more axial-ligated. All the zinc tetrapyrroles in benzene complexed with pyridine to form 5-coordinated (1:1) complexes, not 6-coordinated bis-adducts. The observed equilibrium constants were consistent with the energy changes of the complexation calculated from molecular modeling.

Introduction

In a photosynthetic apparatus, (bacterio)chlorophylls (=(B)Chls) interact with peptides to make a variety of complexes which play important roles including light-harvesting and energy/electron transfer.[1] One of the major interactions is complexation of the central magnesium with the imidazole moiety in histidine of peptides as an axial ligand. The relationship between the coordinated abilities and the naturally occurring tetrapyrrole ligands is of interest both chemically and biologically but no report of work is available to our knowledge.

In natural systems, magnesium of (B)Chls is found to be 5-coordinated, L-MgN_4, from vibrational spectral and X-ray crystallographic analyses.[1] In vitro, (B)Chls are usually coordinated with one axial ligand to give 5-coordinated Mg; 6-coordinated Mg was also observed in highly coordinatable solvents such as pyridine and tetrahydrofuran.[2] However, no unequivocal evidence is available for the presence of 4-coordinated Mg in (B)Chls. Therefore, the complexation of an axial-ligand free (B)Chl with a ligand was not directly observed and the coordinated ability of the central Mg could only be estimated from the exchange reaction, L_1-$MgN_4 + L_2 \rightarrow L_2$-$MgN_4 + L_1$.[3]

On the other hand, synthetic zinc complexes prepared by replacement of the central Mg in (B)Chls with Zn, zinc (bacterio)pheophytins (=Zn-(B)Phes) are good structural and functional models for naturally occurring (B)Chls.[4,5] Recently, it was found that Zn-BPhe-*a* is used instead of BChl-a in a special photosynthetic bacterium.[6] In zinc porphyrins, both the 4- and 5-coordinated zincs (ZnN_4 and L-ZnN_4) were isolated and direct measurements of the coordinated ability were made, $ZnN_4 + L \rightarrow L - ZnN_4$.[7,8] Moreover, a few 6-coordinated zinc porphyrins (L_1L_2-MgN_4) were proposed in the solid state from the crystallographic analyses,[9] but no experimental evidence was submitted for the presence of 6-coordinated zinc in a solution.

Here we report on synthesis of zinc pheophytin analogues and the relationship between the coordinated abilities and

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the tetrapyrrole ligands, which showed that the central zinc was coordinated with pyridine to form 5-coordinated (1:1) complexes, not 6-coordinated bis-adducts, in benzene solution.

Results and discussion

Synthesis of zinc tetrapyrroles

Methyl pyropheophorbide-*a* (**7**) was prepared by modifying chlorophyll-*a*.[10] Transformation[4,10,11] of the 3-vinyl group of **7** to acetyl or ethyl group and/or reduction of the 13-keto carbonyl group of **7** to methylene group, followed by zinc metallation afforded zinc chlorins **1–4** possessing/lacking the $3^1/13^1$-oxo group (Scheme 1). Methyl bacteriopyropheophorbide-*a* (**8**) was prepared from bacteriochlorophyll-*a*.[11] The metal-free compound **8** was zinc-metallated to give zinc bacteriochlorin **5**, which was the 7,8-dihydrogenated form of **4** (Scheme 2). 17,18-Oxidation[12] of zinc chlorin **4** afforded the corresponding zinc porphyrin **6**. All the synthetic tetrapyrroles were characterized by ^{1}H NMR, infrared, visible, mass spectra and/or elemental analyses. Zinc tetrapyrroles **1–6** were purified by several recrystallizations and the pure samples were used for the following spectroscopic analyses.

Visible spectral change in complexation of zinc chlorins with pyridine

In dry benzene, synthetic zinc chlorins **1–4** (= ZnN_4) showed several sharp absorption peaks in the visible region, which were characteristic of monomeric species. Benzene cannot coordinate to the central zinc and the monomeric species in the solution were 4-coordinated zinc complexes without axial ligand. To the solution (ca. 10 μM), highly coordinatable pyridine (= Py) was added dropwise at 25 °C, which induced visible spectral changes (see Fig. 1). In the initial stage of the titration, the absorption spectra were greatly changed, but finally the spectral change was so small that it was undetectable ([Py] > 200 μM). Clear isosbestic points indicated the presence of two species in the visible spectral change: either an equilibrium between ZnN_4 and $PyZnN_4$ (5-coordination, n = 1 in eq (1)) or between ZnN_4 and Py_2ZnN_4 (6-coordination, n = 2). In the dry benzene solution, equilibrium constant (K) in the complexation of ZnN_4 with Py was derived by eq (2).

$$ZnN_4 + nPy \rightarrow (Py)_nZnN_4 \quad (1)$$

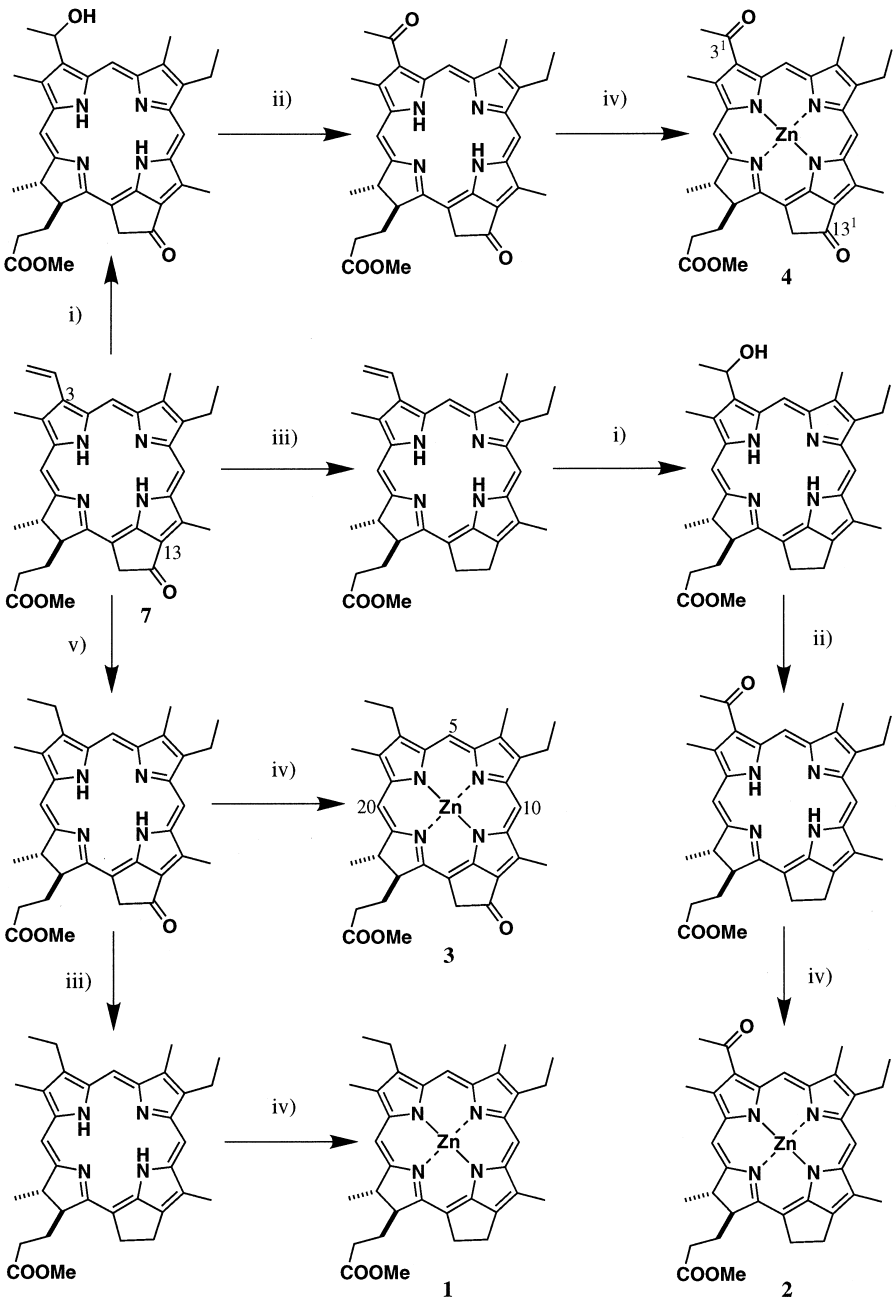
$$K = [(Py)_nZnN_4]/[ZnN_4][Py]^n = \alpha/(1-\alpha)\{[Py]_0 - n\alpha[ZnN_4]_0\}^n \quad (2)$$

The values of the ratio (α) of the complexation were determined from the change of the absorbance (Abs) at any wavelength: $\alpha = (Abs - Abs_{initial})/(Abs_{final} - Abs_{initial})$,[8] at $[Py]_{initial} = 0$ μM and $[Py]_{final} > 200$ μM. The values of K were calculated for all concentrations of added pyridine at any wavelength. Use of n = 1 afforded almost the same estimated K-value for each concentration (the error was within 10%), while use of n = 2 gave large deviation for the K-values. The results strongly supported that all the synthetic zinc chlorins **1–4** were bonded with a single pyridine in the solution to form 1:1 coordinated complexes. The K-values at several wavelengths and the average K are listed in Table 1. These data show that addition of oxo group to the chlorin ligand enhanced the equilibrium constant. The 3/13-keto carbonyl group(s) withdrew the electron on the central zinc through the chlorin π-plane to produce more Lewis acidic and axial-ligated Zn. These substituents on the peripheral position affected the coordinated ability of the central zinc. The substituent effect was consistent with previous reports on complexation of metalloporphyrins with *N*-ligands.[13]

^{1}H NMR spectral change in complexation of zinc chlorin 3 with pyridine

In benzene-d_6, zinc chlorin **3** was monomeric and 4-coordinated zinc complex from the ^{1}H NMR spectrum ([**3**] = 1.7 mM). During the titration of pyridine, ^{1}H NMR spectra were measured at 25 °C. The signals of protons at the *meso*-positions (5-, 10-, and 20-positions) of **3** were shifted to the lower field in the course of the titration (see Figure 2(A)). Addition of one equivalent of pyridine almost induced saturation in the low field shift, indicating 1:1 complexation of **3** with pyridine. The K-values were obtained from eq (2) [n = 1]. The values of α were determined from the change in the chemical shifts (δ) at the *meso*-positions, $\alpha = (\delta - \delta_{initial})/(\delta_{final} - \delta_{initial})$. Each K was calculated for all concentrations of added pyridine. The average K-values were 26000, 23000, and 21000 from change of $\delta_{5\text{-H}}$, $\delta_{10\text{-H}}$, and $\delta_{20\text{-H}}$, respectively, which were similar to the value from visible spectral change described above (= 29900).

During the titration, all the δ-values of pyridine also increased (see Figure 2(B)). Below one equivalent ($[Py]_0 < 1.7$ mM), all the signals of pyridine were shifted to a higher field than those of uncoordinated pyridine and this high field shift decreased in the order, $\delta_{o-H} > \delta_{m-H} > \delta_{p-H}$. These results led to the nitrogen of pyridine coordinating to the central zinc and pyridine protons being affected by the ring current of the chlorin π-moiety. These titration curves in pyridine proton signals fit the theoretical eq (2) [n = 1], which proved to be about $2-3\times10^4$ for the K-value from data of the *o*−, *m*−, and *p*-H. These values were consistent with



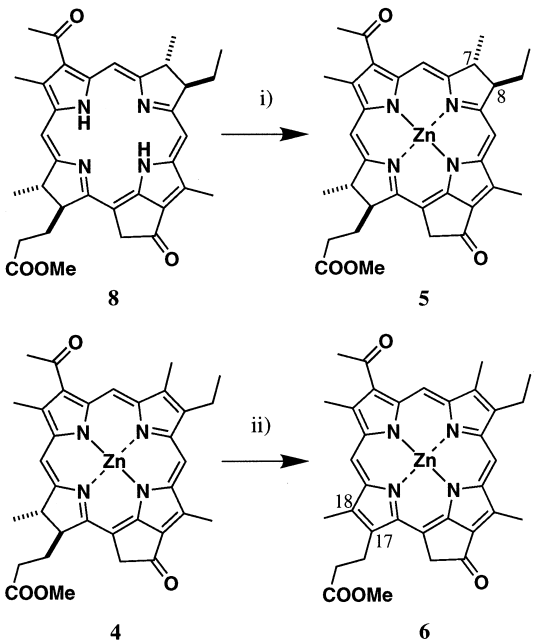
Scheme 1. i) HBr–AcOH (55°C), CH_2N_2/Et_2O; ii) Pr_4NRuO_4–Me(O)N$(CH_2CH_2)_2O/CH_2Cl_2$; iii) $NaBH_4$–TFA/CH_2Cl_2 (0 °C); iv) $Zn(OAc)_2{\cdot}2H_2O$/MeOH–CH_2Cl_2; v) H_2/Pd–C/CH_3COCH_3.

those obtained from visible and ^1H NMR spectral changes of zinc chlorin **3** (vide supra).

Job's plots[14] drawn from change of the chemical shifts at the above six proton signals (see Fig. 3) clearly supported a 1:1 complex of **3** with pyridine in benzene-d_6 ([**3**] + [Py] = 1.2 mM).

Complexation of zinc tetrapyrroles with pyridine

Visible absorption spectral change of dry benzene solution of zinc tetrapyrroles **4–6** by titration of pyridine led to the equilibrium constant K for the 1:1 complexation as described above. The values of K were estimated to be 49600, 65300, and 21400 for **4**, **5**, and **6**, respectively



Scheme 2. i) $Zn(OAc)_2{\cdot}2H_2O$/MeOH–CH_2Cl_2; ii) DDQ/ CH_3 $COCH_3$.

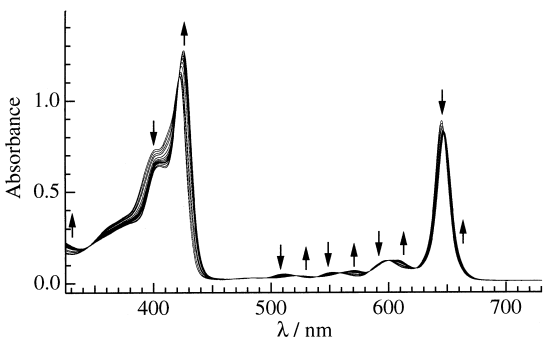
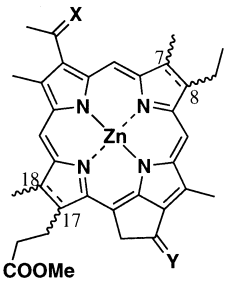


Figure 1. Absorption spectral change in dry benzene solution of zinc chlorin **3** (=12 μM) by addition of pyridine (=0–340 μM) at 25 °C. Arrows show the direction of absorbance change with addition of pyridine.

(see Table 1). The decrease in π-conjugation of the tetrapyrrole ligand (porphyrin **6** > chlorin **4** > bacteriochlorin **5**) enhanced these values. The result is explained by an electronic effect (vide supra) and/or the following coordination process. Considering that all the substituents at the peripheral positions were the same in **4**–**6**, the flexibility of the tetrapyrrole π-plane affected the coordinated ability of the central zinc. In flexible bacteriochlorin **5**, the 4-coordinated zinc in the nearly square planar complex (ZnN_4) moved in an axial direction and formed the pyramidal 1:1 complex with pyridine ($PyZnN_4$) more easily than in **4** and **6**. The central zinc of rigid porphyrin **6** was fixed more tightly to give the mono-adduct with more difficulty than those of **4** and **5**.

Table 1. Equilibrium constant K for 1:1 complexation of zinc tetrapyrroles **1–6** with pyridine in benzene at 25 °C



X	Y	7–8	17–18	K/M^{-1}(λ/nm)	Average K
1 H_2	H_2	C=C	CH–CH (17*S*,18*S*)	12500 (396) 13100 (403) 14200 (503)	13300 ± 700
2 O	H_2	C=C	CH–CH (17*S*,18*S*)	26400 (402) 26900 (414) 26100 (512) 27900 (641)	26800 ± 600
3 H_2	O	C=C	CH–CH (17*S*,18*S*)	28600 (404) 31200 (511) 30500 (550) 29400 (645)	29900 ± 900
4 O	O	C=C	CH–CH (17*S*,18*S*)	46200 (409) 49000 (432) 52700 (439) 50500 (672)	49600 ± 2000
5 O	O	CH–CH (7*R*,8*R*)	CH–CH (17*S*,18*S*)	64000 (356) 64600 (394) 68300 (559) 64300 (578)	65300 ± 1500
6 O	O	C=C	C=C	24800 (423) 19100 (430) 22300 (552) 19400 (621)	21400 ± 2100

Estimation of complexation constants by molecular modeling

Energy-minimized molecular structures of zinc tetrapyrroles (ZnN_4) and the complexes ($PyZnN_4$) with a pyridine as an axial ligand were estimated by MM+/PM3 calculation.[15] To suppress flexibility in the substituents at the peripheral position, the structures of **1–6** for the molecular modeling were slightly changed; both the 8-ethyl and the 17-propionate groups were substituted with a methyl group. The energy difference ΔE between the optimized ZnN_4 and $PyZnN_4$ was calculated. Figure 4 shows that the calculated ΔE is linearly correlated with log K induced by experimental results

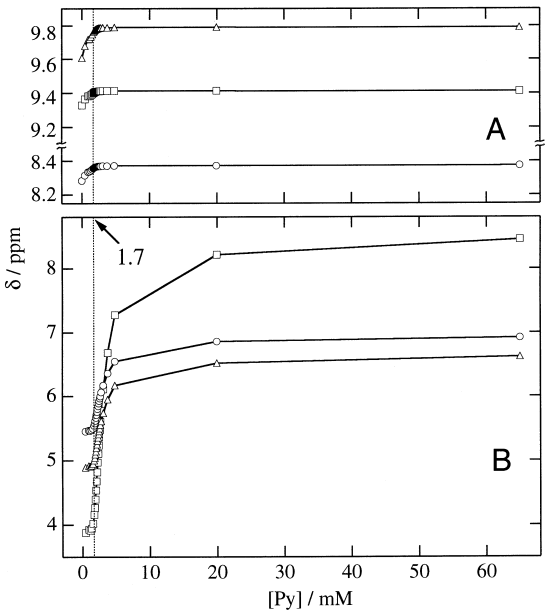


Figure 2. Change of chemical shifts (δ) of (A) 5-H (□), 10-H (△) and 20-H (○) of zinc chlorin **3** (=1.7 mM) and (B) *o*-H (□), *m*-H (△), and *p*-H (□) of additive pyridine (=0–65 mM) in benzene-d_6 at 25 °C.

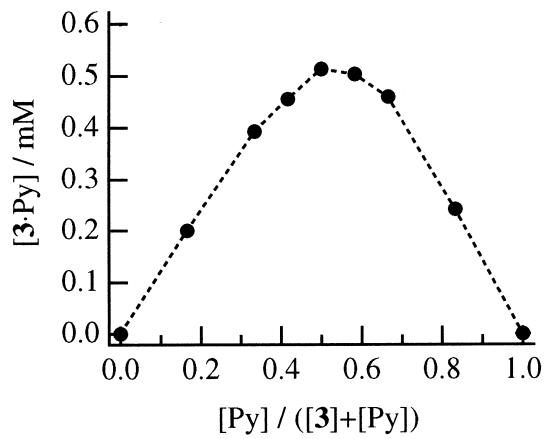


Figure 3. Job's plot of [**3**·Py] estimated from chemical shift change of the *ortho*-proton in pyridine ([**3**]+[Py]=1.2 mM, in benzene-d_6, at 25 °C).

from the visible spectral change, which is expected from the equation of $\Delta G = -RT \ln K$. As a result, the equilibrium constants of the coordination are well estimated by the molecular modeling. The same molecular modeling was performed in naturally occurring BChls-*a*/*b*/*c*/*d*/*e*/*f*/*g* and Chls-*a*/*b*/c_1/*d*. The order of ΔE in these (B)Chls (=magnesium complexes) was similar to that in zinc complexes **1–6** as expected, and the K-values in the

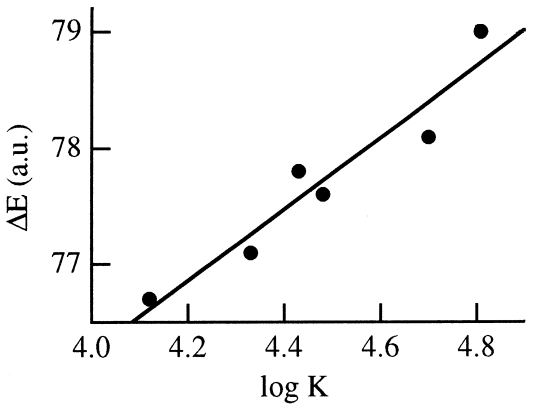


Figure 4. Dependency of ΔE calculated from molecular modeling with log K observed from visible spectral change.

coordination were estimated in the following orders: BChl-*a*/*b* (3-acetyl-bacteriochlorin) > BChl-*g* (3-vinyl-bacteriochlorin) > Chl-*a* (3-vinyl-chlorin) > Chl-c_1 (3-vinyl-porphyrin); Chl-*b* (3-vinyl-7-formyl) > Chl-*d* (3-formyl-7-methyl) > Chl-*a* (3-vinyl-7-methyl) > BChl-*d* (3-(1-hydroxyethyl)-7-methyl-chlorin); BChl-*f* (7-formyl-20-hydrogen) > BChl-*d* (7-methyl-20-hydrogen) = BChl-*e* (7-formyl-20-methyl) > BChl-*c* (7-methyl-20-methyl).

Experimental

All melting points were measured with a Yanagimoto micro melting apparatus and were uncorrected. Visible and infrared absorption spectra were measured with Hitachi U-3500 and Shimadzu FTIR-8600 spectrophotometers, respectively. ^{1}H NMR spectra were measured with a Bruker AC-300 spectrometer; δs are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) as an internal reference. Mass spectra were recorded on a JEOL HX-100 spectrometer; FAB-MS samples were dissolved in $CHCl_3$ and *m*-nitrobenzyl alcohol was used as the matrix. The elemental analyses were performed at the Microanalysis Center of Kyoto University.

Methyl pyropheophorbide-*a* (**7**),[10] methyl mesopyropheophorbide-*a* (3-Et-**7**),[16] methyl bacteriopheophorbide-*d* (3-CH(OH)Me-**7**)[10] and methyl bacteriopyropheophorbide-*a* (**8**)[11] were prepared according to the reported procedures. All synthetic procedures were done in the dark. Flash column chromatography (FCC) was performed with silica gel (Merck, Kieselgel 60, 9385). Dry benzene for visible spectral analysis was prepared as follows. After stirring with H_2SO_4 overnight, benzene was separated, washed with water, refluxed with CaH_2 under nitrogen, distilled

and stored over molecular sieves 3A. Benzene-d_6 for ^{1}H NMR spectral analysis was purchased (99.6% d, CEA) and used without further purification.

General procedures

Hydration of 3-vinyl group. 3-Vinyl-chlorin (0.1 mmol) was dissolved in 30% hydrogen bromide in acetic acid (25 mL) and then stirred at 55 °C under nitrogen for 4 h. The solution was poured into cold H_2O, extracted with $CHCl_3$, washed with aq. 4% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated in vacuo. The residue was treated with an ethereal solution of excess diazomethane without moisture. The solution was stirred for 2 h and evaporated and the resulting residue was purified by FCC with 7–10% Et_2O–CH_2Cl_2 and recrystallization from CH_2Cl_2–hexane to give 3^1-epimeric mixtures ($3^1R/3^1S = 1/1$) of the corresponding chlorin possessing 1-hydroxyethyl group at the 3-position.

Oxidation of 3^1-hydroxyl group. 4-Methylmorpholine-*N*-oxide (24 mg) and tetrapropylammonium perruthenate (10 mg) were added to a dry CH_2Cl_2 (20 mL) solution of chlorin possessing 1-hydroxyethyl group at the 3-position (0.1 mmol) and stirred at room temperature under nitrogen. After disappearance of the 3^1-hydroxy-chlorin from VIS and TLC analyses (ca. 1.5 h), the reaction mixture was poured into H_2O. The aqueous layer was extracted with several portions of CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by FCC with Et_2O–CH_2Cl_2 and recrystallization from CH_2Cl_2–hexane to give the corresponding chlorin possessing 3-acetyl group.

Reduction of 13-keto carbonyl group. Sodium borohydride (87 mg) was added to ice-chilled trifluoroacetic acid (3.4 mL) under nitrogen. To the stirred mixture was added a CH_2Cl_2 solution of a 13^1-oxo-chlorin (0.1 mmol). After stirring overnight at room temperature, the reaction mixture was poured into H_2O. The aqueous layer was extracted with several portions of CH_2Cl_2 and the combined organic layers were washed with aq. 4% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by FCC with CH_2Cl_2 and recrystallization from CH_2Cl_2–hexane to give the corresponding 13^1-desoxo-chlorin.

Zinc metallation. A saturated methanol solution with $Zn(OAc)_2{\cdot}2H_2O$ (1 mL) was added to a CH_2Cl_2 (10 mL) solution of metal-free chlorin (10 mg) and stirred at room temperature under nitrogen. After VIS and TLC analyses showed the metal-free chlorin had disappeared, the reaction mixture was poured into aq. 4% $NaHCO_3$, and stirred for 10 min. White precipitates produced were removed by filtration and the filtrate was dried over Na_2SO_4 and evaporated. The resulting residue was recrystallized from CH_2Cl_2–hexane to give the corresponding zinc complex. Several recrystallizations afforded an analytically pure sample to serve for spectroscopic analyses.

Synthesis of zinc methyl 13^1-desoxo-mesopyropheophorbide-*a* (1)

Methyl mesopyropheophorbide-*a*[16] was reduced by $NaBH_4$–TFA in CH_2Cl_2 at 0 °C[17] to give the 13^1-desoxo compound as dark purple crystals in 82% yield; mp 204–208 °C (lit.[18] 197 °C). MS (FAB) found: *m*/*z* 536. Calcd for $C_{34}H_{40}N_4O_2$: M^+, 536.

The above metal-free compound was zinc-metallated for 10 min to give the title compound as metallic dark purple crystals in 82% yield; mp 213–216 °C; VIS (C_6H_6) $\lambda_{max} = 615$ (ε, 59900), 570 (6120), 542 (3520), 504 (6390), 403 (239000), 382 (55200) nm and (CH_2Cl_2) $\lambda_{max} = 613$ (relative intensity, 18), 567 (2.6), 541 (0.65, sh), 504 (2.9), 401 (100), 378 (23, sh) nm; IR (KBr) 1730 (17^2-COO), 1616 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) $\delta = 9.62$, 9.57, 8.70 (each 1H, s, 5-, 10-, 20-H), 4.73, 4.68 (each 1H, dt, $J = 17$, 5 Hz, 13^1-CH_2), 4.58 (1H, dq, $J = 2$, 7 Hz, 18-H), 4.40 (1H, dt, $J = 8$, 2 Hz, 17-H), 3.90, 3.89 (each 2H, q, $J = 8$ Hz, 3-, 8-CH_2), 3.88 (2H, m, 13-CH_2), 3.54, 3.46, 3.43, 3.36 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.66–2.75, 2.51–2.61, 2.31–2.46, 2.11–2.24 (each 1H, m, 17-CH_2CH_2), 1.83 (3H, d, $J = 7$ Hz, 18-CH_3), 1.76_3, 1.73_5 (each 3H, t, $J = 8$ Hz, 3^1-, 8^1-CH_3). MS (FAB) found: *m*/*z* 598. Calcd for $C_{34}H_{38}N_4O_2{}^{64}Zn$: M^+, 598. Anal found: C; 67.21, H; 6.39, N; 9.14. Calcd for $C_{34}H_{38}N_4O_2Zn{\cdot}1/2H_2O$: C; 67.04, H; 6.45, N; 9.19.

Synthesis of zinc methyl 3-acetyl-3-devinyl-13^1-desoxo-pyropheophorbide-*a* (2)

The synthetic route of the title compound (**7**→→→→**2**) was preliminarily reported by Tamiaki et al.[21] and the detailed procedures and the spectral data are described here.

Methyl pyropheophorbide-*a* (**7**)[4,10,19] was reduced by $NaBH_4$–THF in CH_2Cl_2 at 0 °C[22] to give the 13^1-desoxo compound as a black solid in 81% yield (lit.[22] 71 %); mp 240–244 °C. MS (FAB) found: *m*/*z* 534. Calcd for $C_{34}H_{38}N_4O_2$: M^+, 534.

The above 3-vinyl compound was hydrated to give 3^1-hydroxy compound as a black solid in 71% yield; mp 165–170 °C; VIS (CH_2Cl_2) $\lambda_{max} = 640$ (rel. 25), 586 (2.5), 541 (1.5, sh), 526 (1.8), 498 (8.6), 490 (7.5, sh), 405 (81, sh), 395 (100) nm; ^{1}H NMR ($CDCl_3$) $\delta = 10.18/17$, 9.61, 8.93 (each 1H, s, 5-, 10-, 20-H), 6.68/65 (1H, q, $J = 7$ Hz, 3-CH), 4.95, 4.82 (each 1H, ddd, $J = 4$, 7,

16 Hz, 13^1-CH_2), 4.67 (1H, dq, $J=2$, 7 Hz, 18-H), 4.50 (1H, dt, $J=9$, 2 Hz, 17-H), 4.09 (2H, m, 13-CH_2), 3.88 (2H, q, $J=8$ Hz, 8-CH_2), 3.60, 3.58, 3.52, 3.45 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.76–2.87, 2.53–2.68, 2.32–2.45, 2.13–2.28 (each 1H, m, 17-CH_2CH_2), 2.23 (3H, d, $J=7$ Hz, 3^1-CH_3), 1.86/1.84 (3H, d, $J=7$ Hz, 18-CH_3), 1.77 (3H, t, $J=8$ Hz, 8^1-CH_3), −3.45 (1H, s, NH (another NH was too broad to be observed)). MS (FAB) found: m/z 552. Calcd for $C_{34}H_{40}N_4O_3$: M^+, 552.

The above 3^1-hydroxy compound was oxidized to give 3-acetyl compound as a black solid in 71% yield (1% Et_2O–CH_2Cl_2 for FCC); mp 221–225 °C; VIS (CH_2Cl_2) $\lambda_{max}=657$ (rel. 27), 603 (4.0), 544 (3.9), 508 (11), 411 (100) nm; ^{1}H NMR($CDCl_3$) $\delta=10.42$, 9.47, 8.98 (each 1H, s, 5-, 10-, 20-H), 4.91, 4.79 (each 1H, ddd, $J=3$, 7, 16 Hz, 13^1-CH_2), 4.62 (1H, dq, $J=2$, 7 Hz, 18-H), 4.46 (1H, dt, $J=7$, 2 Hz, 17-H), 4.05 (2H, m, 13-CH_2), 3.82 (2H, q, $J=8$ Hz, 8-CH_2), 3.76, 3.59, 3.47, 3.42, 3.36 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.73–2.84, 2.57–2.67, 2.17–2.40 (1H + 1H + 2H, m, 17-CH_2CH_2), 1.84 (3H, d, $J=7$ Hz, 18-CH_3), 1.84, (3H, d, $J=7$ Hz, 18-CH_3), 1.75 (3H, t, $J=8$ Hz, 8^1-CH_3), −2.89 (1H, br, NH (another NH was too broad to be observed)). MS (FAB) found: m/z 550. Calcd for $C_{34}H_{38}N_4O_3$: M^+, 550.

The above metal-free compound was zinc-metallated for 1.5 h to give the title compound as metallic purple crystals in 84% yield; mp 132–135 °C; VIS (C_6H_6) $\lambda_{max}=641$ (ε, 46200), 596 (6100), 515 (5300), 514 (5400, sh), 414 (123000), 382 (28300, sh) nm and (CH_2Cl_2) $\lambda_{max}=639$ (rel. 25), 595 (4.7, sh), 550 (1.4, sh), 511 (6.3), 411 (100) nm; IR (KBr) 1736/1712 (17^2-COO), 1649 (3-C=O), 1616 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) $\delta=9.89$, 9.38, 8.77 (each 1H, s, 5-, 10-, 20-H), 4.75, 4.65 (each 1H, dt, $J=16$, 6 Hz, 13^1-CH_2), 4.58 (1H, dq, $J=2$, 7 Hz, 18-H), 4.41 (1H, dt, $J=7$, 2 Hz, 17-H), 3.91 (2H, m, 13-CH_2), 3.76 (2H, q, $J=8$ Hz, 8-CH_2), 3.55, 3.53 3.40, 3.26, 3.05 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.66–2.78, 2.53–2.63, 2.32–2.42, 2.18–2.27 (each 1H, m, 17-CH_2CH_2), 1.86 (3H, d, $J=7$ Hz, 18-CH_3), 1.71 (3H, t, $J=8$ Hz, 8^1-CH_3). MS (FAB) found: m/z 612. Calcd for $C_{34}H_{36}N_4O_3{}^{64}Zn$: M^+, 612. Anal found: C; 60.00, H; 5.35, N; 7.98. Calcd for $C_{34}H_{36}N_4O_3Zn\cdot CH_2Cl_2$: C; 60.10, H; 5.48, N; 8.01.

Synthesis of zinc methyl mesopyropheophorbide-*a* (3)

Methyl mesopyropheophorbide-*a*[16] was zinc-metallated for 1.5 h to give the title compound as purple crystals in 76% yield; mp 144–147 °C (lit.[4] 144–148 °C); VIS (C_6H_6) $\lambda_{max}=645$ (ε, 76100), 599 (9410), 551 (3250), 423 (96300), 402 (60200), 380 (30400, sh) nm; see ref. 4 for IR and ^{1}H NMR data. MS (FAB) found: m/z 612. Calcd for $C_{34}H_{36}N_4O_3{}^{64}Zn$: M^+, 612. Anal found: C; 66.21, H; 6.04, N; 8.83. Calcd for $C_{34}H_{36}N_4O_3Zn$: C; 66.50, H; 5.91, N; 9.12.

Synthesis of zinc methyl 3-acetyl-3-devinyl-pyropheophorbide-*a* (4)

Methyl bacteriopheophorbide-*d*[10,19] was oxidized by Pr_4RuO_4−Me(O)N$(CH_2CH_2)_2O$ in CH_2Cl_2[11] to give the 3-acetyl compound as a black solid in 75% yield (5% Et_2O–CH_2Cl_2 for FCC); mp 264–267 °C (lit.[20] 264–265 °C); VIS (CH_2Cl_2) $\lambda_{max}=683$ (rel. 52), 623 (7.4), 547 (11), 514 (12), 417 (100), 384 (79) nm; ^{1}H NMR ($CDCl_3$) $\delta=10.00$, 9.61, 8.78 (each 1H, s, 5-,10-, 20-H), 5.34, 5.18 (each 1H, d, $J=20$ Hz, 13^1-CH_2), 4.56 (1H, dq, $J=2$, 7 Hz, 18-H), 4.37 (1H, dt, $J=9$, 2 Hz, 17-H), 3.73 (2H, q, $J=8$ Hz, 8-CH_2), 3.72, 3.66, 3.61, 3.30 (3H + 3H + 3H + 6H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.54–2.78, 2.25–2.37 (each 2H, m, 17-CH_2CH_2), 1.84 (3H, d, $J=7$ Hz, 18-CH_3), 1.72 (3H, t, $J=8$ Hz, 8^1-CH_3), −1.75 (1H, br, NH (another NH was too broad to be observed)). MS (FAB) found: m/z 564. Calcd for $C_{34}H_{36}N_4O_4$: M^+, 564.

The above metal-free compound was zinc-metallated for 2 h to give the title compound as green crystals in 95% yield; mp 137–140 °C; VIS (C_6H_6) $\lambda_{max}=672$ (ε, 51900), 620 (7300), 569 (4000), 526 (2760), 432 (68000), 384 (31400, sh) nm and (CH_2Cl_2) $\lambda_{max}=669$ (rel. 73), 618 (11), 568 (6.0), 525 (3.7), 429 (100), 404 (66, sh), 380 (54, sh) nm; IR (KBr) 1736 (17^2-COO), 1668 (3/13-C=O), 1615 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) $\delta=9.44$, 8.93, 8.52 (each 1H, s, 5-,10-, 20-H), 4.90, 4.87 (each 1H, d, $J=20$ Hz, 13^1-CH_2), 4.49 (1H, dq, $J=2$, 7 Hz, 18-H), 4.28 (1H, dt, $J=8$, 2 Hz, 17-H), 3.65 (2H, q, $J=8$ Hz, 8-CH_2), 3.54, 3.37, 3.25, 3.02, 2.70 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.42–2.61, 2.13–2.36 (each 2H, m, 17-CH_2CH_2), 1.87 (3H, d, $J=7$ Hz, 18-CH_3), 1.67 (3H, t, $J=8$ Hz, 8^1-CH_3). MS (FAB) found: m/z 626. Calcd for $C_{34}H_{34}N_4O{}^{64}Zn$: M^+, 626. Anal found: C; 64.40, H; 5.45, N; 8.87. Calcd for $C_{34}H_{34}N_4O_4Zn\cdot 1/2H_2O$: C; 64.10, H; 5.54, N; 8.79.

Synthesis of zinc methyl bacteriopyropheophorbide-*a* (5)

Methyl bacteriopyropheophorbide-*a* (**8**, 20 mg)[11] was zinc-metallated in refluxing $CHCl_3$ (10 mL) and MeOH saturated with $Zn(OAc)_2\cdot 2H_2O$ (10 mL) for 3 h (no metallation occurred by stirring at room temperature as described above) to give the title compound as a dark blue solid in 66% yield; mp 193–196 °C; VIS (C_6H_6) $\lambda_{max}=774$ (ε, 144000), 706 (12400, sh), 561 (28800), 435 (4450, sh), 394 (62700), 356 (95200) nm and (CH_2Cl_2) $\lambda_{max}=771$ (rel. 100), 700 (13, sh), 553 (27), 433 (9.3, sh), 391 (58), 351 (91) nm; IR (KBr) 1736 (17^2-COO), 1650 (3/13-C=O), 1604 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) $\delta=8.82$, 8.41, 8.26 (each 1H, s, 5-,10-, 20-H), 5.03, 4.88

(each 1H, d, $J=20$ Hz, 13^1-CH_2), 4.21 (2H, dq, $J=3$, 7 Hz, 7-, 18-H), 4.03 (2H, m, 8-, 17-H), 3.57, 3.47, 3.45, 3.11 (each 3H, s, 2-, 3^1-, 12-CH_3, $COOCH_3$), 2.42–2.54, 2.17–2.39, 1.89–2.07 (1H + 3H + 2H, m, 8-CH_2, 17-CH_2CH_2), 1.70 (6H, d, $J=7$ Hz, 7-, 18-CH_3), 0.94 (3H, t, $J=7$ Hz, 8^1-CH_3). MS (FAB) found: m/z 628. Calcd for $C_{34}H_{36}N_4O_4{}^{64}Zn$: M^+, 628.

Synthesis of zinc methyl 3-acetyl-3-devinyl-protopyropheophorbide-*a* (6)

To an acetone (30 mL) solution of zinc methyl 3-acetyl-3-devinyl-pyropheophorbide-*a* (**1**, 21 mg) was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (11 mg).[12] After stirring for 5 min, the solution was poured into H_2O, extracted with CH_2Cl_2 and washed with aq. 4% $NaHCO_3$. The organic phase was dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by FCC with 13–14% Et_2O–CH_2Cl_2 and recrystallization from CH_2Cl_2–MeOH to give the title compound as a purple solid in 63% yield; mp 267–270 °C; VIS (C_6H_6) $\lambda_{max}=621$ (ε, 32700), 568 (7700), 430 (234000), 418 (110000, sh) nm and (CH_2Cl_2) $\lambda_{max}=619$ (rel. 12), 568 (2.4), 425 (100), 414 (53, sh) nm; IR (KBr) 1736 (17^2-COO), 1695/1670 (3/13-C=O) cm^{-1}; ^1H NMR ($CDCl_3$) $\delta=10.56$, 9.79, 9.60 (each 1H, s, 5-,10-, 20-H), 5.37 (2H, s, 13^1-CH_2), 4.02 (2H, q, $J=8$ Hz, 8-CH_2), 3.89 (2H, t, $J=8$ Hz, 17-CH_2), 3.79, 3.78, 3.72, 3.63, 3.40, 3.31 (each 3H, s, 2-, 3^1-, 7-, 12-, 18-CH_3, $COOCH_3$), 2.85 (2H, t, $J=8$ Hz, 17^1-CH_2), 1.84 (3H, t, $J=8$ Hz, 8^1-CH_3). MS (FAB) found: m/z 624. Calcd for $C_{34}H_{32}N_4O_4{}^{64}Zn$: M^+, 624.

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