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Synthesis and Self-Assembly of Zinc Methyl Bacteriopheophorbide-*f* and its Homolog

Hitoshi Tamiaki,* Masanobu Kubo and Toru Oba†

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

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Abstract—Zinc methyl bacteriopheophorbide-*f*, zinc 3-(1-hydroxyethyl)-7-formyl-chlorin, and its 3^1-demethyl derivative were prepared by modification of naturally occurring chlorophyll-*b*. The former complex was a 3^1-epimeric mixture and separated to pure stereoisomers by HPLC, while such a separation was unnecessary for the latter possessing the 3-hydroxymethyl group. The absolute configuration at the 3^1-position of the separated samples was determined by transformation of the 7-formyl to the 7-methyl group because the 3^1-stereochemistry of zinc methyl bacteriopheophorbide-*d* (3-(1-hydroxyethyl)-7-methyl-chlorin) has been confirmed. All the synthetic zinc chlorins self-aggregated in 0.5% (v/v) THF–hexane as well as in 6% (v/v) THF–water to form oligomers which absorbed longer-wavelength lights by the J-aggregation than the monomers in THF. Diastereomeric control in the self-aggregation of the 3^1-epimers was observed. The 3^1-demethyl compound interacted more tightly in the self-aggregates than the 3^1-epimers. Comparing with the corresponding 7-methyl analogs, zinc 7-formyl-chlorins have red-shifted Soret and blue-shifted Q_y bands in both the monomeric and aggregated states. Visible, circular dichroism and resonance Raman spectra showed that the supramolecular structures of self-aggregates of the 7-formyl and 7-methyl derivatives were similar and highly ordered, and were built-up by 13-C=O···H(3-CHR)O···Zn and π–π interaction of the chlorin chromophores. The coordinatable 7-formyl group did not disturb the special bondings of the composite molecules in the self-aggregates.

Introduction

Photosynthetic reaction centers consist of pigment–protein complexes and the supramolecular structures are similar in all the present species.[1,2] In contrast, several types of light-harvesting antenna systems exist in plants, algae and bacteria;[3,4] inner- and extramembraneous apparatus, and pigment–protein and pigment–pigment interaction. Green bacteria have quite a unique antenna system on the inner surface of the cytoplasmic membrane and the extra-membraneous antennae are called chlorosomes.[3,5] The core part is constructed by self-aggregation of chlorophyllous pigments without any protein scaffolds,[5–8] which is completely different from other core parts of structurally determined antenna systems.[9–11] The self-aggregative chlorophylls in chlorosomes are mixtures of several homologs of bacteriochlorophyll(BChl)s-*c*, *d* and *e* (see Fig. 1),[12] whereas other antennae usually have a sole structural formula as the chlorophyllous pigment in an apparatus. Such a diversity in the molecular structures, variation in R^7, R^8, R^{12}, R^{20}, R-groups and the absolute configuration at the 3^1-position as shown in the left structure of Fig. 1, affects the chlorosomal structures and functions[5,7] and the ratios among the composite pigments are easily changed by the culturing conditions.[13–15] Self-assemblies of BChls-*c* and *d* (7-methyl-chlorins, R^7=Me) were investigated in the natural and artificial systems;[5–7] far fewer reports are available for in vivo and in vitro self-aggregates of BChl-*e* (7-formyl-chlorin, R^7=CHO).[16–25] Moreover, self-aggregation of BChl-*f* which is the 20-demethyl analog of BChl-*e* has never been investigated because of the unavailability of BChl-*f* in any bacteria.[26]

We earlier reported that zinc analogs of naturally occurring magnesium complexes are good model compounds for BChls-*c* and *d*.[6,27–36] Here we report the synthesis of zinc methyl 3^1-*R*- and 3^1-*S*-bacteriopheophorbides-*f* (**Zn-1R** and **Zn-1S**), derivatives of one BChl-*f* homolog possessing R^8=Et and R^{12}=Me and the 3^1-demethyl homolog **Zn-2** by modification of chlorophyll-*b* which is easily available from spinach, and the self-aggregation of the synthetic zinc 3^1-hydroxy-7-formyl-13^1-oxo-chlorins **Zn-1/2** in a non-polar organic solvent as well as in an aqueous organic solution. We particularly investigated the supramolecular structures of self-aggregates of the synthetic zinc 7-formyl-chlorins compared with those of the corresponding 7-methyl analogs by their visible, circular dichroism, and resonance Raman spectra.[37]

Keywords: aggregation; chlorophyll; stereochemistry; supramolecular chemistry.

* Corresponding author. Fax: +81-77-561-2659;
e-mail: tamiaki@se. ritsumei.ac.jp

† Present address: Department of Applied Chemistry, Faculty of Engineering, Utsunomiya University, Utsunomiya, Tochigi 321-8585, Japan

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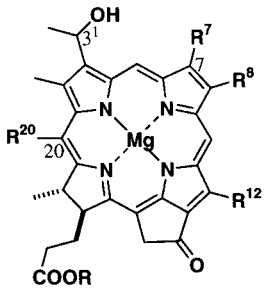
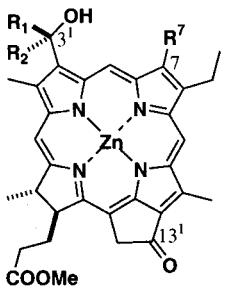


BChl-*c*: $R^7=R^{20}=Me$
BChl-*d*: $R^7=Me$, $R^{20}=H$
BChl-*e*: $R^7=CHO$, $R^{20}=Me$
BChl-*f*: $R^7=CHO$, $R^{20}=H$



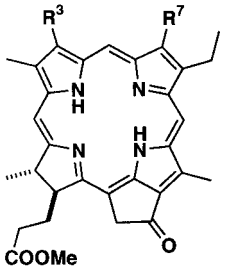
Zn-1R: $R_1=Me$, $R_2=H$, $R^7=CHO$
Zn-1S: $R_1=H$, $R_2=Me$, $R^7=CHO$
Zn-2: $R_1=R_2=H$, R =CHO
Zn-3R: $R_1=R^7=Me$, $R_2=H$
Zn-3S: $R_1=H$, $R_2=R^7=Me$
Zn-4: $R_1=R$ =H, $R^7=Me$

Figure 1. Chlorosomal bacteriochlorophylls (left) and their model zinc complexes (right).

Results and Discussion

Synthesis of 3^1-hydroxy-7-formyl-chlorins

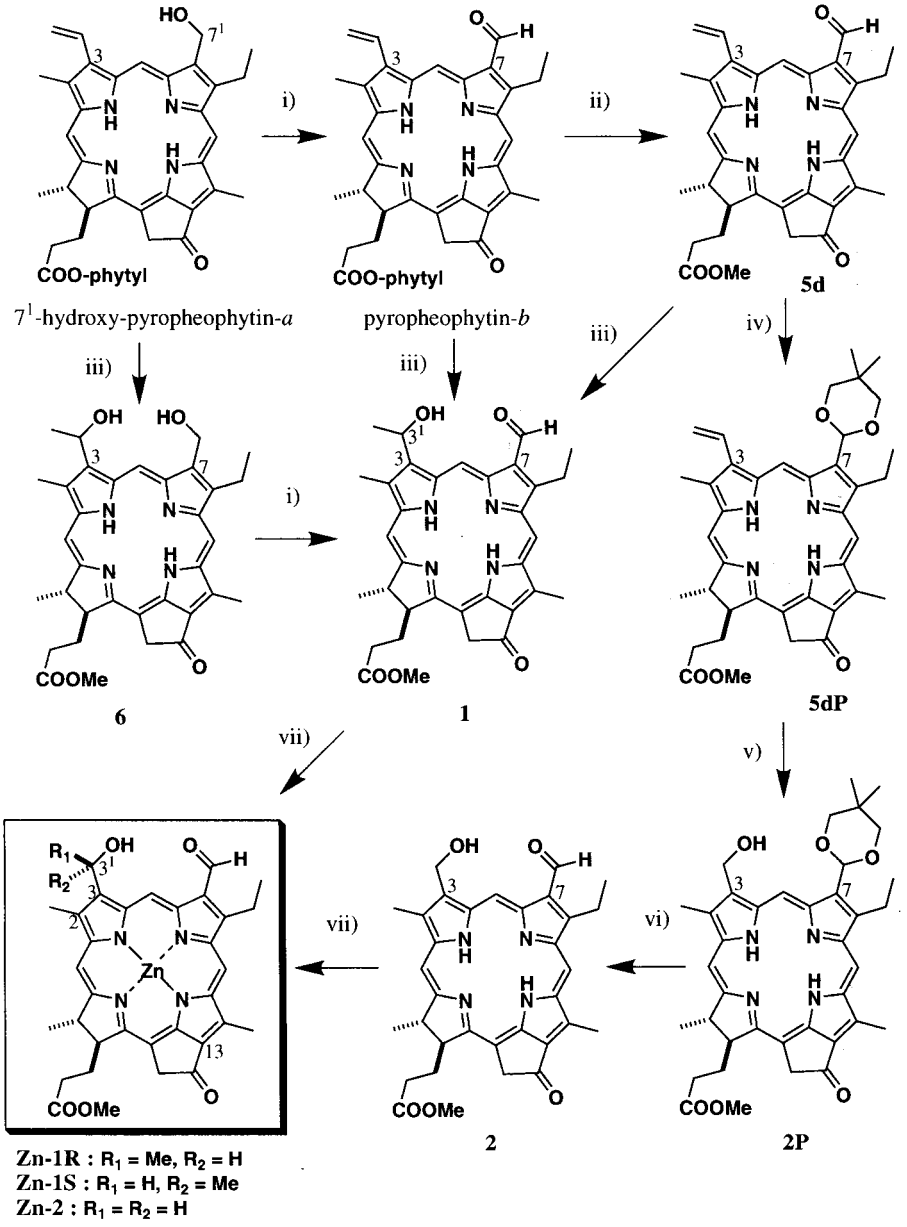
Preparation of methyl bacteriopheophorbide-*f* (1, see Fig. 2) has been reported by Risch et al.[38] and Simpson and Smith.[39] Both the synthetic routes were based on hydration of the 3-vinyl group (R^3=CH=CH_2) in methyl pyropheophorbide-*b* (**5d**) by treatment of hydrogen bromide in acetic acid and successive hydrolysis of the resulting bromide. In the former, heating at 180°C for 8 min was required and the latter report gave no detailed procedures. First, we examined the synthetic procedures described in the former report[38] but could not find desired **1** in the reaction mixture. For the hydration, we next used procedures similar to those used in hydration of the 3-vinyl group in methyl pyropheophorbide-*a* (**5a**, heating at 55°C for 3 h),[40] but again were unable to get **1**. Both the reaction mixtures after hydration were very complex, and no proton signals characteristic of the 7-formyl group were observed at around δ=11 ppm in the ^{1}H NMR spectra of either mixture. These results indicate that the 7-CHO reacted with HBr or AcOH under the above conditions. Therefore, we attempted hydration of the 3-vinyl group in methyl 7^1-hydroxy-pyropheophorbide-*a* (**5b**) possessing 7-CH_2OH which was a synthetic precursor of 7-formyl-chlorin **5d**. Heating of 3-vinyl-chlorin **5b** in 30% HBr–AcOH for 3 h and successive treatment by ice water and diazomethane gave desired methyl 7^1-hydroxy-bacteriopheophorbide-*d* (**6**) possessing 3-CH(OH)Me in 10–20% yield after purification by silica gel chromatography. The yield was lower than that in similar hydration of 3-vinyl-7-methyl-chlorin **5a** (50%[40]–86%[33]). The other products gave no proton signal characteristic of 7-CH_2OH at around δ=5.7 ppm in the ^{1}H NMR spectra, indicating that the 7^1-position was also attacked by HBr or AcOH under the reaction conditions. To suppress formation of the by-products, the hydration conditions were changed to be mild; the reaction temperature in HBr–AcOH was lowered. Reaction of 3-vinyl-7-hydroxymethyl-chlorin **5b** at 20°C for 12 h gave the corresponding 3-(1-hydroxyethyl)-chlorin **6** more efficiently (42%).



1: R^3=CH(OH)Me, R^7=CHO
2: $R^3=CH_2OH$, R^7=CHO
3: R^3=CH(OH)Me, $R^7=CH_3$
4: $R^3=CH_2OH$, $R^7=CH_3$
5a: R^3=CH=CH_2, $R^7=CH_3$
5b: R^3=CH=CH_2, $R^7=CH_2OH$
5c: R^3=CH=CH_2, $R^7=CH_2OMe$
5d: R^3=CH=CH_2, R^7=CHO
5e: $R^3=R^7=CH_2OH$
5f: R^3=CHO, $R^7=CH_2OH$
5g: $R^3=R^7$=CHO
5h: R^3=COMe, R^7=CHO
6: R^3=CH(OH)Me, $R^7=CH_2OH$
7: R^3=CH(OH)Me, $R^7=CH_2OMe$

Figure 2. Methyl bacterio- and pyropheophorbides.

Methyl 7^1-hydroxy-pyropheophorbide-*a* (**5b**) was given by transformation of the phytyl ester in 7^1-hydroxy-pyropheophytin-*a* (see left structure in top row of Scheme 1) which was prepared by pyrolysis of 7^1-hydroxy-pheophytin-*a* easily isolated from spinach extracts treated with *t*-$BuNH_2BH_3$.[41] The transesterification by H_2SO_4 in MeOH initially gave methyl ester **5b** and the prolonged reaction also afforded 7-CH_2OMe in **5c**. Over-reaction could be avoided by careful check of the reaction mixture. However, methyl 7^1-hydroxy-bacteriopheophorbide-*d* (**6**) could also be prepared by reaction of 7^1-hydroxy-pyropheophytin-*a* with HBr–AcOH at 20°C followed by H_2O and CH_2N_2 (see Scheme 1), because the phytyl ester was hydrolyzed under the acidic conditions. The successive reactions were simpler than the separated reactions (phytyl→methyl ester and 3-vinyl→3-(1-hydroxyethyl)) and the yield of the former (43%) was higher than the overall yield of the latter (35%). The 7-hydroxymethyl group in **6** was selectively oxidized by pyridinium dichromate (PDC)[26] to give the corresponding 7-formyl-chlorin **1** (79%). The selective oxidation was dependent upon the reaction time. Prolonged reaction induced oxidation of the 1-hydroxyethyl at the 3-position to afford 3-acetyl-7-formyl-chlorin **5h**. The process of the oxidation must be checked by measurement of visible spectra of the reaction mixture; the Soret peak moved from 420 nm to 440 nm by oxidation on the



Scheme 1. Synthesis of zinc methyl bacteriopheophorbide-*f* (**Zn-1**) and its homolog (**Zn-2**): (i) PDC; (ii) H_2SO_4/MeOH; (iii) HBr/AcOH, CH_2N_2; (iv) $HOCH_2CMe_2CH_2OH$–*p*-TsOH; (v) OsO_4–$NaIO_4$, *t*-$BuNH_2BH_3$; (vi) HCl; (vii) $Zn(OAc)_2$.

7-position and the Q_y peak moved from 650 nm to 680 nm by oxidation on the 3-position.

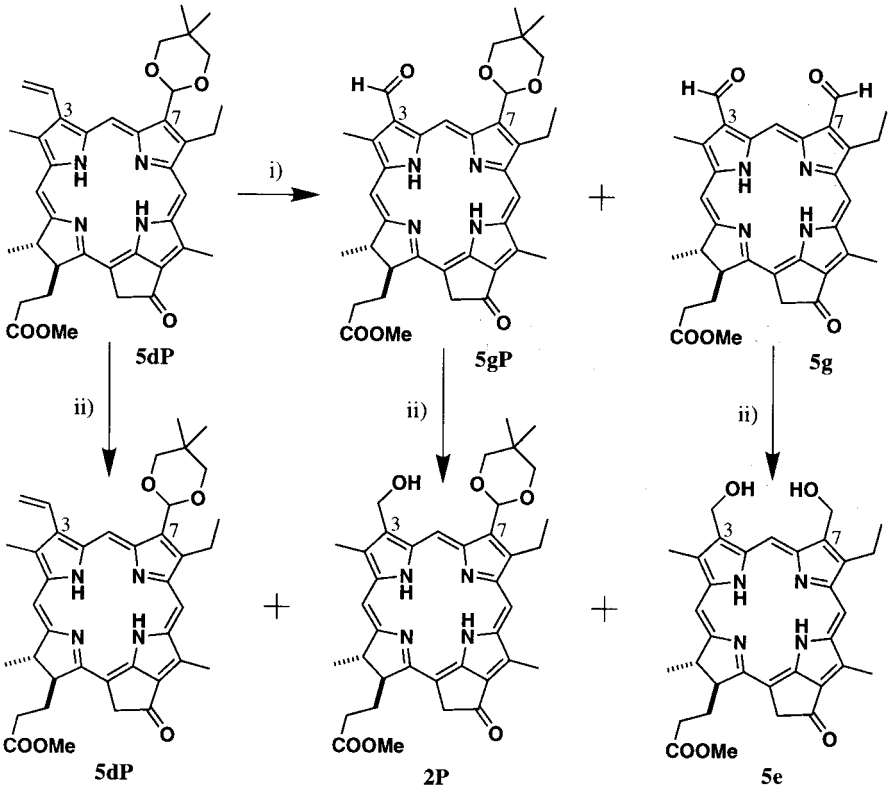
Mild addition of HBr to the 3-vinyl group in 7-hydroxymethyl-chlorin was effective for the preparation of **1** described above. Then, we attempted a similar mild hydration of the 3-vinyl-7-formyl-chlorin. Methyl pyropheophorbide-*b* (**5d**) was treated with 30% HBr–AcOH at 20°C for 15 h, followed by water and diazomethane to give **1** in 62% yield. The reaction at 20°C was effective for addition of HBr to the 3-vinyl group in any chlorins possessing a reactive functional group. Methyl ester **5d** was easily synthesized by transformation (H_2SO_4 in MeOH) of the phytyl ester in pyropheophytin-*b* (88%) which was readily prepared by oxidation (PDC in C_6H_6) of 7^1-hydroxy-pyropheophytin-*a* (75%). Neither the oxidation nor the transesterification afforded any over-reacted products for a prolonged reaction time. The synthetic route initiated by oxidation via **5d** is more effective for preparation of **1** from 7^1-hydroxy-pyropheophytin-*a* in both procedures and total yield (41%=0.75×0.88×0.62×100) than the route initiated by hydration via **6** (34% yield=0.43×0.79×100).

Finally, hydration of pyropheophytin-*b* by treatment of 30% HBr–AcOH at 20°C for 15 h, followed by H_2O and CH_2N_2 was examined and gave **1** in 55% yield. The yield of the direct hydration in the phytyl ester is the same as the overall yield via methyl ester **5d** (55%=0.88×0.62×100, vide supra). The former synthetic procedures was simpler than the latter. Therefore, preparation of **1** by initial oxidation of the 7^1-hydroxy-pyropheophytin-*a* (from spinach) followed by acidic hydration (and concomitant acidic hydrolysis of phytyl ester) and successive methyl-esterification of the resulting pyropheophytin-*b* was the best of the synthetic

routes examined here. The addition of HBr to the 3-vinyl group was non-diastereoselective, which led to formation of an epimeric mixture of 3^1R (1*R*)/3^1S (1*S*)=1/1 (from ^{1}H NMR spectral analysis). It is noted that benzene as the reaction solvent is important for oxidation of the 7-hydroxymethyl group by PDC; any by-products (not determined) which were absorbed at around 705 nm were produced for oxidation in dichloromethane and could not be separated from the desired 7-formyl-chlorin. Moreover, all the reactions must be done in the dark; the light promoted formation of any by-products in HBr-addition.

Synthetic chlorin **1** is a 3^1-epimeric mixture and **1R** and **1S** must be separated to get a pure sample. To avoid the separation, the 3^1-demethyl derivative **2** was prepared. Analog **2** has a hydroxymethyl group at the 3-position and the 3^1-carbon is not asymmetric. Since we have already reported that a similar 3-hydroxymethyl-chlorin **4** (7-methyl analog of **2**) is a good model for several naturally occurring esters of bacteriopheophorbides-*c* and *d* possessing 3-CH(OH)Me,[29] **2** must be useful as a model of **1** (vide infra). To prepare analog **2**, we first tried to oxidize selectively 3,7-bis(hydroxymethyl)-chlorin **5e** which was readily afforded by transformation[42] of the 3-vinyl group of **5b** to the 3-hydroxymethyl group via the 3-formyl group as in **5f**. Treatment of the diol **5e** with PDC gave a mixture of desired 7-formyl-chlorin **2** (major) and the regioisomeric 3-formyl-chlorin **5f** (minor) as the single oxidation products. These regioisomers were difficult to separate. Next, we examined the selective reduction of 3,7-diformyl-chlorin **5g** prepared by oxidation of 3-formyl-7-hydroxymethyl-chlorin **5f** (the intermediate in the synthesis of the above diol **5e**, vide supra) but the reduction by *t*-BuNH_2BH_3 gave a mixture of desired 3-hydroxymethyl-chlorin **2** (major) and the regioisomeric 7-hydroxymethyl-chlorin **5f** (minor).

We then adopted the following synthetic route for preparation of **2**: transformation of the 3-vinyl to the 3-hydroxyethyl group after protection of the 7-formyl group. The formyl group at the 7-position of methyl pyropheophorbide-*b* (**5d**) was protected with alcohols by action of an acid to give the acetal. When methanol was used as the alcohol, the resulting dimethyl acetal was so unstable[39] that the acetal bonds were completely cleaved during a usual workup such as washing the dichloromethane solution with water. In the case of protection by propylene glycol, the cyclic acetal was slightly deprotected in the workup. The acetal by neopentyl glycol is stable[43] enough to be purified by the workup and silica gel chromatography and the protected acetal **5dP** was given in 99% yield (see Scheme 1). Oxidation of the vinyl group at the 3-position of the **5dP** by OsO_4–$NaIO_4$ in aq. AcOH and THF gave the formyl group and concomitantly there was partial deprotection of the acetal. To suppress the undesired deprotection, the reaction must be quenched with water before the oxidation has been completed. The reaction mixture contained three chlorins: 3-formyl-7-acetal-protected-formyl **5gP**:3,7-diformyl **5g**:3-vinyl-7-acetal-protected-formyl **5dP**=72:16:12 from the ^{1}H NMR spectral analysis (see Scheme 2). These compounds could not be separated easily and the mixture was used for the following reduction without further separation. The mixture was reduced by *t*-BuNH_2BH_3 to afford a mixture of 3-hydroxymethyl-7-acetal-protected-formyl-, 3,7-bis(hydroxymethyl)- and 3-vinyl-7-acetal-protected-formyl-chlorins (**2P**, **5e** and **5dP**) which were easily separated by silica gel chromatography because of the



Scheme 2. Synthesis of mono-alcohol **2P** by modification of 3-vinyl-chlorin **5dP**: (i) OsO_4–$NaIO_4$; (ii) *t*-BuNH_2BH_3.

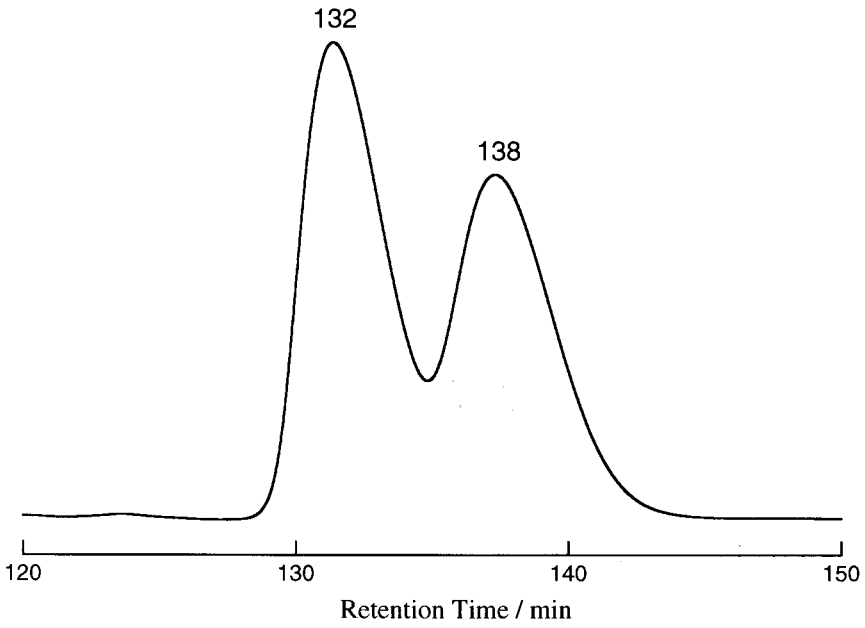


Figure 3. HPLC chromatogram of a 1:1 3^1-epimeric mixture of zinc methyl bacteriopheophorbide-*f* (**Zn-1R/S**); Cosmosil $5C_{18}$-AR, 6 mm ϕ×250 mm, Nacalai Tesque, MeOH/H_2O=4/1, 1.0 mL/min.

difference in the number of hydroxyl groups in the molecule. The acetal protection of the separated mono-alcohol **2P** was cleanly cleaved by stirring aq. 1% HCl in acetone to give desired 3-hydroxymethyl-7-formyl-chlorin **2**.

The metal-free chlorins **1** and **2** were zinc-metallated[30] to give zinc complexes **Zn-1** and **Zn-2**, respectively, after purification by HPLC. All the synthetic chlorins were characterized by their ^{1}H NMR, visible, infra-red and mass spectra.

Separation and structure determination of 3^1-epimeric chlorins

Risch and his colleagues have reported[38] that a 3^1-epimeric mixture of metal-free chlorin **1** could be separated by reversed-phase HPLC and the absolute configuration was

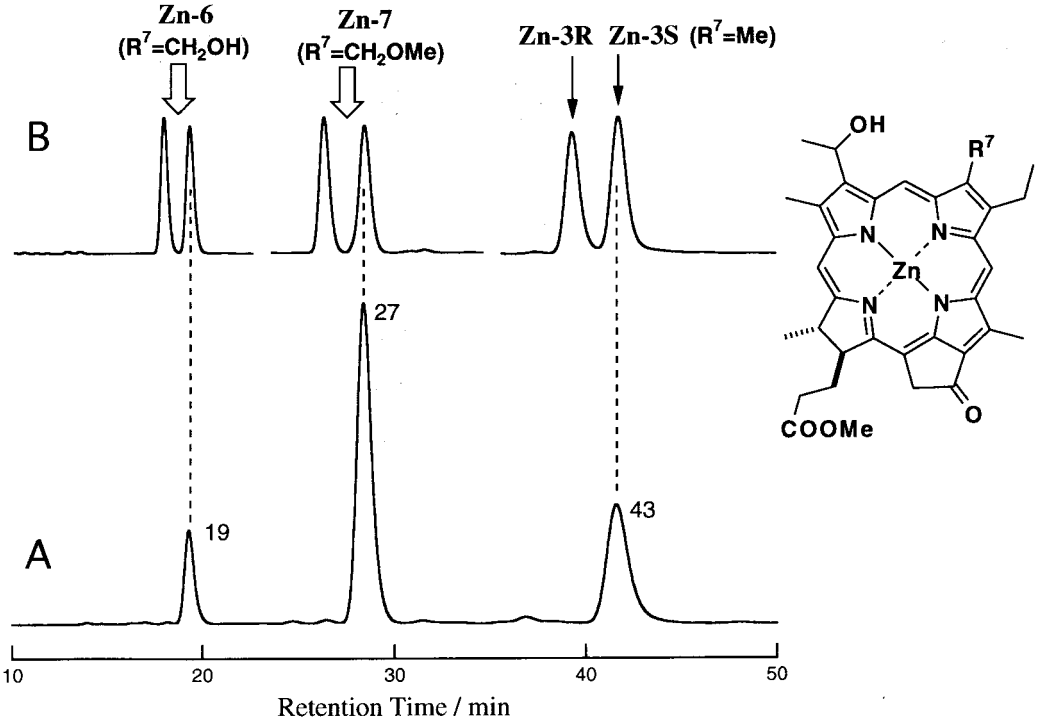


Figure 4. HPLC chromatograms of (A) a reaction mixture prepared by reduction of epimerically pure zinc methyl bacteriopheophorbide-*f* (**Zn-1**, 138-min eluted band in Fig. 3) with $NaCNBH_3$ in MeOH and (B) 1:1 3^1-epimeric mixtures of zinc methyl 7^1-hydroxy-, 7^1-methoxy- and 7^1-unsubstituted-bacteriopheophorbides-*d* (**Zn-6**, **Zn-7** and **Zn-3**).

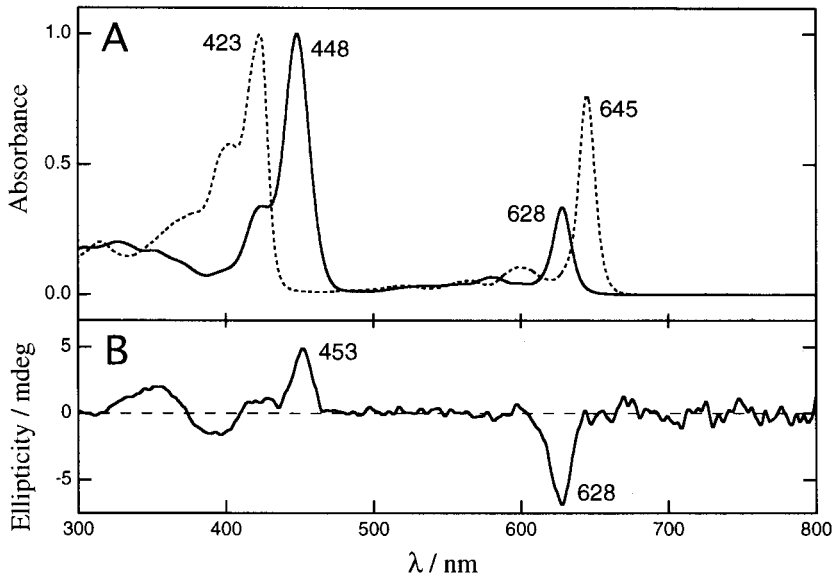


Figure 5. Visible (A) and CD spectra (B) of zinc methyl 3^1*R*-bacteriopheophorbide-*f* (**Zn-1R**, solid line) and visible spectra of zinc methyl 3^1*R*-bacteriopheophorbide-*d* (**Zn-3R**, broken line of (A)) in THF.

estimated by the ^{1}H NMR spectra of the ester derivatives of a *N*-protected chiral phenylalanine; comparison of the chemical shifts at the *meso*-protons in the D-Phe-**1** derivative was made with those in the corresponding ester of structurally determined 3^1-epimeric methyl bacteriopheophorbide-*d*. According to the reported procedures (an ODS column, MeOH/H_2O=4/1),[38] we tried to separate a 1:1 mixture of **1** but obtained ill-resolved bands by a single run of HPLC; the separation ratio was 0.5 at most. A 3^1-epimeric mixture of zinc complex **Zn-1** was partially separated after a single HPLC run (Cosmosil 5C_{18}-AR, 6 mm ϕ×250 mm, Nacalai Tesque, MeOH/H_2O=2/1, 1.5 mL/min); the retention times were 132 and 138 min and the separation ratio was about 0.8 (see Fig. 3). After the repeated runs, each 3^1-epimer was obtained in pure form. To determine the absolute configuration of the asymmetric 3^1-position unambiguously, the 3^1-epimerically separated zinc chlorins **Zn-1** were transferred to stereochemistry-determined zinc methyl bacteriopheophorbide-*d* (**Zn-3**).[33] According to the procedures reported by Scheumann and his colleagues,[44] the second HPLC-eluted epimer of **Zn-1** was reduced by $NaCNBH_3$ in methanol to give three products as shown in a HPLC chromatogram of the reaction mixture (Fig. 4A). Comparison with the authentic samples (Fig. 4B) showed the 19-min band was 7-hydroxymethyl-chlorin **Zn-6**, the 27-min band was 7-methoxymethyl-chlorin **Zn-7**, and the 43-min was zinc methyl 3^1*S*-bacteriopheophorbide-*d* (**Zn-3S**). Therefore, the 3^1-configuration of the first (second) eluted band in **Zn-1** was determined to be *R*(*S*)-form. During the reduction, no epimerization was observed and the *R*-epimers of all the zinc chlorins examined were eluted faster than the corresponding *S*-epimers under the HPLC conditions.

Zinc chlorins in a polar organic solvent

Synthetic zinc chlorins were highly soluble in polar organic solvents as tetrahydrofuran (THF), pyridine and so on. These solvent molecules coordinated with the central zinc as an axial ligand to give a penta-coordinated zinc complex. All three complexes **Zn-1R**, **Zn-1S** and **Zn-2** gave almost the same visible spectra in THF (solid line of Fig. 5A). Sharp bands were obtained in 628 nm (Q_y peak) and 448 (Soret peak), indicating that these compounds were monomeric in comparison with reported data of BChl-*e*.[45] The Q_y and Soret peaks of zinc 7-formyl-chlorins are shifted to shorter and longer wavelengths, respectively, than those (645 and 423 nm) of the corresponding zinc 7-methyl-chlorins **Zn-3R**, **Zn-3S** and **Zn-4** in THF (broken line of Fig. 5A). These shifts are observed in naturally occurring chlorophylls: Chl-*b* (7-CHO, 644 and 455 nm) and Chl-*a* (7-Me, 662 and 430 nm) in diethyl ether; BChl-*e* (7-CHO, 647 and 458 nm) and BChl-*c* (7-Me, 660 and 428 nm) in acetone.[46] Interestingly, the blue (red) shift in the Q_y (Soret) peak by **Zn-3R** (7-Me)→**Zn-1R** (7-CHO) is 420 (1300) cm^{-1} which is the same as the shifted value in Chl-*a*→Chl-*b*, and the consistency is due to the structural similarity that all four metal-chlorins are unsubstituted at the 20-position. The ratios of the peak intensities in **Zn-1R**, **Zn-1S** and **Zn-2** were 0.34 for Abs(Q_y)/Abs(Soret), while the ratios in **Zn-3R**, **Zn-3S** and **Zn-4** were 0.77. About half this ratio and the blue (red) shift in the Q_y (Soret) peak are characteristic features in visible spectra of 7-formyl-chlorins, compared with those of 7-methyl-analogs.

The above observation in visible spectra was made with electronic spectra estimated by modeling calculation. Calculation of THF-coordinating **Zn-1R** by successive MM+ and ZINDO/S (HyperChem 5.1)[34] gave the same wavelengths in allowed excitation (S_0→S_n) as those of **Zn-1S** and **Zn-2.** The ZINDO/S calculation based on the molecular structure of THF·**Zn-1R** optimized by the MM+ calculation predicted a HOMO to LUMO transition at 625 nm, in good agreement with the experimental value of 628 nm in THF. The estimated Soret peak position in the calculation (342 nm) was different from the experimentally observed peak at 448 nm. Theoretically calculated

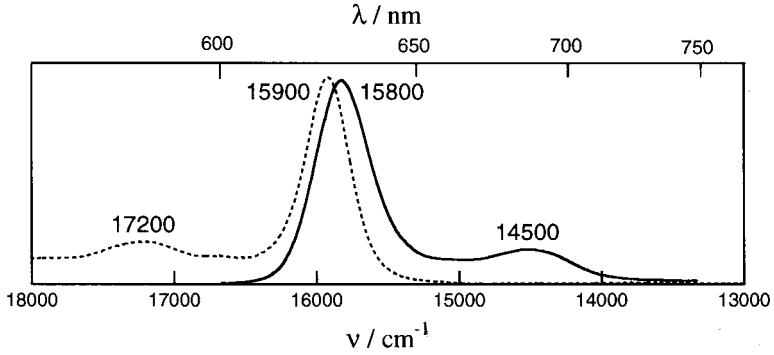


Figure 6. Fluorescence (excited at 448 nm, solid line) and visible spectra (broken line) of zinc methyl 3^1*R*-bacteriopheophorbide-*f* (**Zn-1R**) in THF.

shifts in the peaks by change of 7-Me to 7-CHO were quite consistent with the experimental shifts; calculated and measured red(blue)-shifts in Q_y(Soret) peak were 350(1100) and 420(1300) cm^{-1}, respectively. The decrease in the ratio of observed peak intensities by 7-Me→7-CHO (0.77→0.34, see Fig. 5A) was also qualitatively predictable from the MM+ and ZINDO/S calculation, Int(Q_y)/Int(Soret)=0.18 (7-Me)→0.16 (7-CHO).

Synthetic zinc chlorin **Zn-1R** in THF gave small negative and positive CD peaks in the Q_y and Soret regions, respectively (see Fig. 5B). When **Zn-1R** in THF was excited in the Soret region, intense fluorescence lights were emitted at the region of >600 nm and the emission spectrum was a mirror image of the absorption spectrum (Fig. 6). Both CD spectra and fluorescence emission spectra (excited at 500 nm) of **Zn-1R**, **Zn-1S** and **Zn-2** in THF are almost the same. An additional methyl group and the stereochemistry at the 3^1-position scarcely affected CD or fluorescence spectra as well as visible spectra in THF.

Resonance Raman spectra in THF (excited at 458 nm) showed that all the monomers gave two peaks at 1700–1690 and 1665–1660 cm^{-1} in the region of 1800 to 1600 cm^{-1} (vide infra). The former is assigned to a 13-C=O stretching vibrational band and the latter to 7-CHO stretching, comparing with reported data.[20,27] These values indicate that the two carbonyl groups are free from any interaction.

Zinc chlorins in a non-polar organic solvent

We earlier reported that zinc 3^1-hydroxy-13^1-oxo-chlorins possessing a methyl group at the 7-position as in **Zn-3** and **Zn-4** self-aggregated in non-polar organic solvents to give red-shifted visible absorption peaks.[29,33] In 0.5% (v/v) THF–hexane, visible bands of **Zn-1** and **Zn-2** were shifted to longer wavelengths and were broader than those in THF (Fig. 7), indicating that the zinc chlorins self-aggregated even in the presence of the 7-formyl group in the molecule. Absorption maxima of Q_y peaks in self-aggregates of **Zn-1** and **Zn-2** possessing 7-CHO (673/677 and 714 nm) are situated at shorter wavelengths than those of **Zn-3** and **Zn-4** possessing 7-Me (ca. 700 and 740 nm), respectively. The blue-shift in Q_y peaks of self-aggregates (7-Me→7-CHO) is ascribable to the blue-shift in the monomeric peaks described above. Self-aggregates of **Zn-1R** showed slightly less red-shifted and a sharper Qy peak (λ_{max}=673 nm, Δ=1250 cm^{-1}, broken line of Fig. 7) than those of the epimeric **Zn-1S** (677 nm, 1530 cm^{-1}, dotted line of Fig. 7). Such a diastereoselective control in self-aggregation has been reported in other 3^1-epimers as in **Zn-3R** and **Zn-3S**.[28,32,33] Zinc 3-hydroxymethyl-chlorin **Zn-2** in 0.5% (v/v) THF–hexane showed a more red-shifted Q_y band at 714 nm (thick solid line of Fig. 7) than the corresponding 3-1-hydroxyethyl analogs **Zn-1**. The demethylation at the 3^1-position induced less steric repulsion and stronger interaction among the composite molecules in the self-aggregates because the hydroxyl group is a connecting

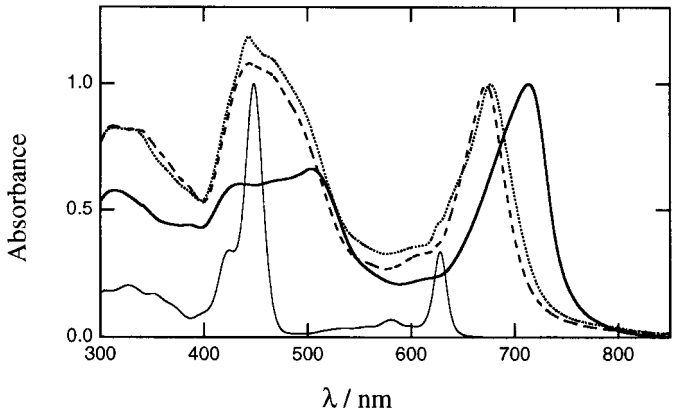


Figure 7. Visible spectra of zinc methyl 3^1*R*-bacteriopheophorbide-*f* (**Zn-1R**, broken line), 3^1*S*-bacteriopheophorbide-*f* (**Zn-1S**, dotted line), and 3^1-demethyl-bacteriopheophorbide-*f* (**Zn-2**, thick solid line) in 0.5% (v/v) THF–hexane and **Zn-2** in THF (thin solid line).

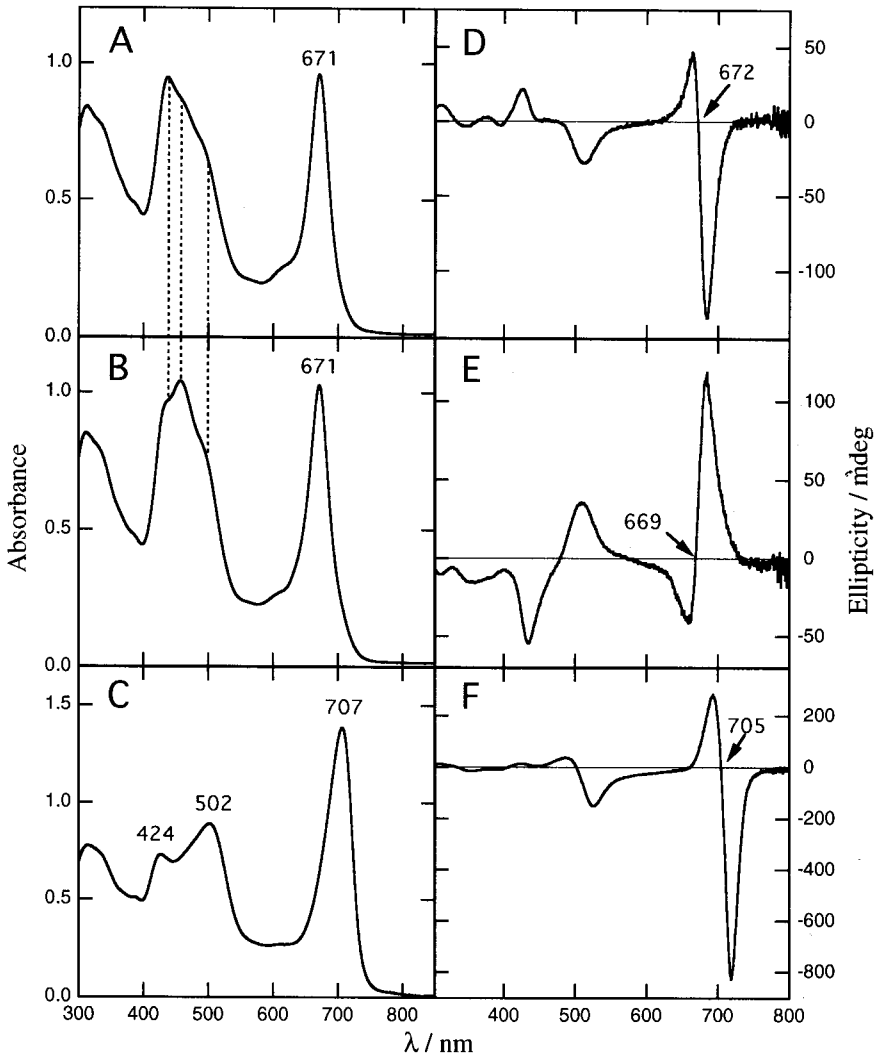


Figure 8. Visible spectra of zinc methyl 3^1*R*-bacteriopheophorbide-*f* (**Zn-1R**, A), 3^1*S*-bacteriopheophorbide-*f* (**Zn-1S**, B), and 3^1-demethyl-bacteriopheophorbide-*f* (**Zn-2**, C) and CD spectra of **Zn-1R** (D), **Zn-1S** (E), and **Zn-2** (F) in 6% (v/v) THF–water.

group as in 13-C═O···HO···Zn.[29] Strong interaction and high oligomerization of **Zn-2** moved the Q_y peak to a longer wavelength and the red-shift by the self-aggregation was 1890 cm^{-1}. The value was comparable to that in **Zn-4** (1990 cm^{-1}),[29] indicating that the self-aggregates of zinc 3^1-hydroxy-7-formyl-13^1-oxo-chlorins would be similar to those of the 7-methyl analogs.

Self-aggregates of **Zn-1/2** have Soret bands in the region of >500 nm, which are situated at longer wavelengths than those of **Zn-3/4** (λ_{max}=440–450 nm).[29,33] The red-shift in the oligomeric Soret peak is due to the red-shift in the monomeric peak. As the oligomeric Soret band, typically, **Zn-2** absorbed the lights at the wide region of 400–550 nm and at least two peaks were observed at 422 and 509 nm, as estimated from the second derivative of the visible spectrum. A relatively large component at around 450–470 nm in **Zn-1** would be due to residual monomer in the solution, which also shows less aggregativity of **Zn-1** than of **Zn-2** described above. Such a broad Soret band with two peaks is characteristic of self-aggregates of zinc 7-formyl-chlorins.

These absorption spectra of **Zn-1/2** in non-polar organic solvents are similar to the in vivo and in vitro self-aggregates of BChl-*e*.[16] This means that the self-aggregates of synthetic zinc chlorins **Zn-1/2** would be good models for natural chlorosomal aggregates of BChl-*e*. The self-aggregates of 7-formyl-chlorins have intense absorbance at around 500 nm which is the region absorbed by carotenoids in a usual photosynthetic apparatus. This would affect the content and variation of carotenoids in BChl-*e* containing chlorosomes (and also living cells) of anoxygenic photosynthetic brown-colored bacteria, e.g. *Chlorobium phaeobacteroides*[47,48] and *phaeovibrioides*.

Zinc chlorins in an aqueous organic solution

Solubility of **Zn-1/2** in non-polar organic solvents is not so high, and the concentrated solution easily changed to be heterogeneous after the preparation and gave some precipitates as in recrystallization. In aqueous organic solutions, zinc chlorins self-aggregate true in non-polar organic solvents.[42,49] In 6% (v/v) THF–water,[50] **Zn-1** and **Zn-2** gave similar red-shifted Q_y peaks at 671 and 707 nm, respectively, (Fig. 8A–C) as in 0.5% (v/v) THF–hexane. These Q_y bands of self-aggregates in the aqueous solution

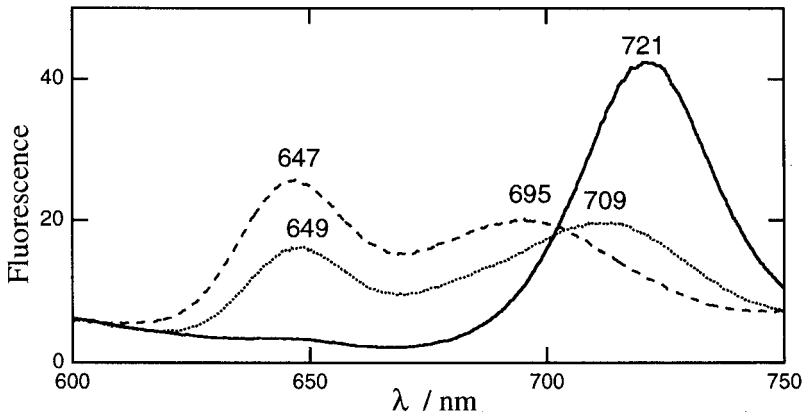


Figure 9. Fluorescence spectra (excited at 500 nm) of zinc methyl 3[1]*R*-bacteriopheophorbide-*f* (**Zn-1R**, broken line), 3[1]*S*-bacteriopheophorbide-*f* (**Zn-1S**, dotted line), and 3[1]-demethyl-bacteriopheophorbide-*f* (**Zn-2**, solid line) in 6% (v/v) THF–water.

were sharper than those in non-polar organic solvents. In the red-shifted Q_y band region, large CD bands were observed (Fig. 8D–F); a large band at a longer wavelength and a small band at a shorter wavelength. The zero-crossing points in the CD spectra are almost the same as the wavelengths of the Q_y absorption maxima. **Zn-1R** gave a reverse S-shaped CD spectrum and **Zn-1S** gave an S-shaped CD spectrum. The difference in the 3[1]-epimers is attributed to diastereoselective control in the self-aggregation.

The fluorescence spectrum of **Zn-1R** in the aqueous solution (excited at 500 nm) shows 647- and 695-nm peaks emitted from monomer (or small aggregates) and aggregates, respectively (broken line of Fig. 9) and self-aggregates of **Zn-1S** gave a longer wavelength emission peak at 709 nm (dotted line of Fig. 9). This difference is also due to the diastereoselective controlling aggregation in the 3[1]-epimers.

The CD couplet of **Zn-2** in the Q_y region is very similar to that of **Zn-1R** (Fig. 8D and F), indicating that both **Zn-1R** and **Zn-2** give similar supramolecular structures in the self-aggregates. Comparing the shapes of visible bands in the Soret region (Fig. 8A and B), **Zn-1S** produced more residual monomer absorbed at around 460 nm than **Zn-1R** in the aqueous solution. Therefore, **Zn-1R** self-aggregated to form more stable supramolecules than **Zn-1S**. The fluorescence spectrum of **Zn-2** gave predominantly a 721-nm band without an emission peak at around 650 nm from the monomer (solid line of Fig. 9), indicating that **Zn-2** self-aggregated more strongly than **Zn-1** and left no residual monomer in the solution.

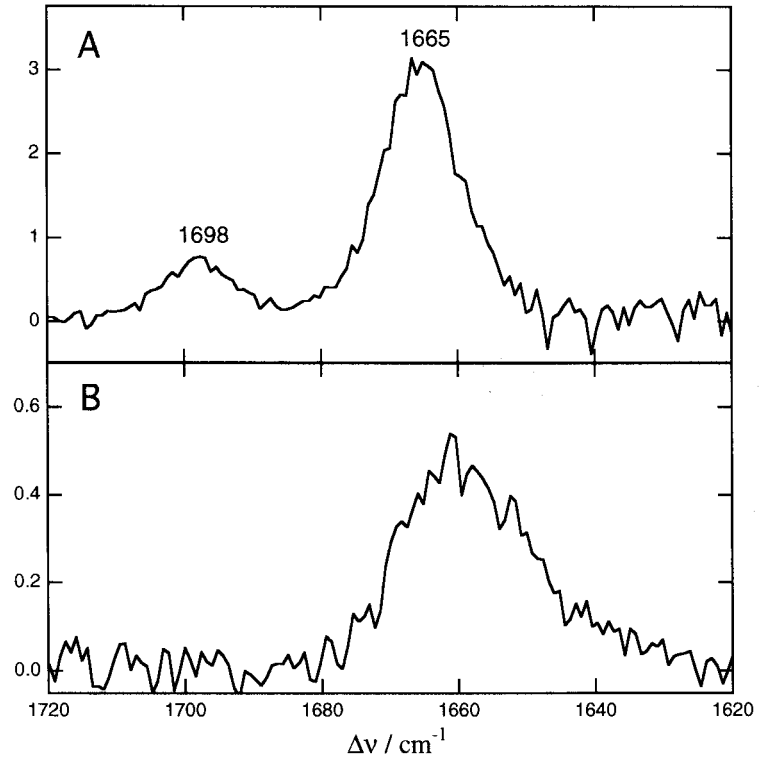


Figure 10. Resonance Raman spectra (excited at 458 nm) of zinc methyl 3[1]-demethyl-bacteriopheophorbide-*f* (**Zn-2**) in THF (A) and in 6% (v/v) THF–water (B).

Resonance Raman spectra (excited at 458 nm) of **Zn-2** were recorded (Fig. 10) to elucidate the supramolecular structure of the self-aggregates. In THF, monomeric **Zn-2** gave two bands at 1698 and 1665 cm^{-1} (Fig. 10A) assignable to 13-C═O and 7-CHO stretching vibrations, respectively, both of which were free from any interaction (vide supra). In 6% (v/v) THF–water, no Raman peak of the 13-carbonyl group at 1700–1690 cm^{-1} was observed in the aqueous solution (Fig. 10B), indicating that the 13-C═O interacted with any functional group in the self-aggregates of **Zn-2**. A broad Raman band at 1680–1640 cm^{-1} was observed in self-aggregated **Zn-2** and was composed of two bands at 1663 and 1652 cm^{-1} from the band-fitting analysis. We have reported that self-aggregates of **Zn-4** in an aqueous solution gave a 1650-cm^{-1} Raman band for the 13-C═O which was specially hydrogen-bonded with 3^1-OH as in 13-C═O···HO···Zn.[35] Based on the similarity in red-shifts of Q_y peaks by self-aggregation of **Zn-2** and **Zn-4** (vide supra), the Raman band at 1652 cm^{-1} is proposed for specially hydrogen-bonded 13-keto-carbonyl stretching in self-aggregated **Zn-2**. The other band at 1662 cm^{-1} is attributed to the stretching of the 7-formyl group, indicating that the 7-CHO in the self-aggregates is free from any interaction. These assignments in the artificial self-aggregates are consistent with the previous report on natural chlorosomal aggregates of BChl-*e*.[20] Therefore, a coordinatable formyl group at the 7-position in **Zn-2** would not interact with any other functional groups of the other molecules in the self-aggregates, and did not disturb the formation of highly ordered supramolecules which were built up mainly by 13-C═O···H(3-CHR)O···Zn and π–π interaction of the chlorin chromophores.

Experimental

General

All of the apparatus used was described in our previous reports.[51,52] Chemical shifts (δ) of ^1H NMR spectra are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) or CHD_2OD (3.30 ppm) as an internal reference.

Zinc metallation of chlorins was done by reported procedures.[30] THF and benzene were distilled from CaH_2 before use. Flash column chromatography (FCC) was performed with silica gel (Merck, Kieselgel 60, 9385). HPLC was performed with a packed ODS column (Gelpack GL-OP100, 6 mm ϕ×150 mm, Hitachi Chemical Co. or Cosmosil 5C_{18}-AR, 6 mm ϕ×250 mm, Nacalai Tesque). Solvents for visible, CD and fluorescence spectra were purchased from Nacalai Tesque (Grade for UV-spectroscopy). All synthetic procedures must be done in the dark!

Synthesis of pyropheophytin-*b*

According to procedures reported by Oba and his colleagues,[41] 7^1-hydroxy-pyropheophytin-*a* was prepared from spinach. Pyridinium dichromate (20 mg) was added to a dry benzene solution (20 mL) of 7^1-hydroxy-pyropheophytin-*a* (18.4 mg) under N_2. After 3-h stirring at room temperature, diethyl ether (20 mL) was added to the reaction mixture, stirred and filtered. The filtrate was washed with brine and dried over Na_2SO_4. The solvents were evaporated and the residue was purified by FCC (2.5% Et_2O–CH_2Cl_2) and recrystallization from CH_2Cl_2 and MeOH to give pyropheophytin-*b* (13.8 mg, 75%); see Ref. 41 for the spectral data.

Synthesis of methyl pyropheophorbide-*b* (5d)

Pyropheophytin-*b* (44 mg) was dissolved in a small amount of dichloromethane (ca. 2 mL), to which methanol (72 mL) was added. To the ice-chilled solution, an ice-cooled methanol solution (4 mL) of sulfuric acid (1 mL) was added dropwise under N_2. After 16-h stirring at room temperature, the reaction mixture was poured into ice-water and CH_2Cl_2, washed with aqueous 4% $NaHCO_3$ solution and dried over Na_2SO_4. Dichloromethane was evaporated to give **5d** (20 mg, 88%); brown solid; mp 218–219°C (lit.[38] 245–246°C); VIS (CH_2Cl_2) λ_{max}=656 (relative intensity, 0.21), 601 (0.07), 530 (0.09), 440 nm (1.00); ^1H NMR ($CDCl_3$) δ=10.86 (1H, s, CHO), 9.97, 9.07, 8.47 (each 1H, s, 5-, 10-, 20-H), 7.88 (1H, dd, *J*=12, 18 Hz, 3-CH), 6.30 (1H, dd, *J*=1, 17 Hz, 3^1-CH-*trans* to C3^1-H), 6.17 (1H, dd, *J*=1, 12 Hz, 3^1-CH-*cis* to C3^1-H), 5.19, 5.03 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.45 (1H, dq, *J*=2, 7 Hz, 18-H), 4.25 (1H, dt, *J*=9, 2 Hz, 17-H), 3.64 (2H, q, *J*=6 Hz, 8-CH_2), 3.66, 3.45, 3.35 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.55–2.70, 2.15–2.40 (each 2H, m, 17-CH_2CH_2), 1.87 (3H, d, *J*=7 Hz, 18-CH_3), 1.61 (3H, t, *J*=8 Hz, 8^1-CH_3), -0.01, -1.98 (each 1H, s, NH). MS (FAB) found: *m/z* 563. Calcd for $C_{34}H_{35}N_4O_4$: MH^+, 563.

Synthesis of methyl bacteriopheophorbide-*f* (1)

Via hydration of 7^1-hydroxy-pyropheophytin-*a*. 7^1-Hydroxy-pyropheophytin-*a* (22 mg) was dissolved in 30% hydrogen bromide in acetic acid (30 mL) and then stirred at 20°C under N_2 for 15 h. CAUTION, reaction in total darkness is very important to suppress formation of any undesired products. The solution was poured into ice-water, extracted with $CHCl_3$, washed with brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was treated with excess ethereal CH_2N_2 solution at 0°C under N_2. After 2-h stirring at room temperature, the solution was poured into brine, washed with brine, and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by FCC (1.7% MeOH–CH_2Cl_2) to give 3^1-epimeric mixtures of methyl 7^1-hydroxy-bacteriopheophorbide-*d* (6, 6.7 mg, 43% yield, (3^1R)/(3^1S)=1/1); black solid (from CH_2Cl_2–hexane); mp 132°C; VIS (CH_2Cl_2) λ_{max}=656 (rel. 0.38), 600 (0.07), 539 (0.07), 508 (0.09), 415 nm (1.00); ^1H NMR ($CDCl_3$) δ=9.86/84, 9.44/43, 8.50/49 (each 1H, s, 10-, 5-, 20-H), 6.42/40 (1H, q, *J*=7 Hz, 3-CH), 5.74 (2H, s, 7-CH_2), 5.16, 5.01 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.43 (1H, dq, *J*=2, 8 Hz, 18-H), 4.21 (1H, dt, *J*=9, 2 Hz, 17-H), 3.75 (2H, q, *J*=7 Hz, 8-CH_2), 3.62/61, 3.59/58, 3.40/38 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.50–2.65, 2.20–2.35 (each 2H, m, 17-CH_2CH_2), 2.13 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.78 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.70 (3H, d, *J*=8 Hz, 18-CH_3), 0.19 (1H, br, NH), -1.89 (1H, s, NH). MS (FAB) found: *m/z* 583. Calcd for $C_{34}H_{39}N_{24}O_5$: MH^+, 583.

To a dry benzene (20 mL) solution of **6** (6.7 mg) was added

pyridinium dichromate (22 mg) and the mixture was stirred at room temperature under N_2 for 3 h. Diethyl ether (40 mL) was added to the mixture, stirred and filtered. The filtrate was washed with brine and dried over Na_2SO_4. After evaporation, the residue was purified by HPLC (retention time=8.4 min, Cosmosil, MeOH, 1.0 mL/min) to give 3^1-epimeric mixtures of **1**[38] (5.3 mg, 79% yield, (3^1R)/(3^1S)=1/1); brown solid (from CH_2Cl_2–hexane); mp 111°C; VIS (CH_2Cl_2) λ_{max}=649 (rel. 0.22), 594 (0.07), 524 (0.08), 436 (1.00), 415 nm (0.45); ^{1}H NMR ($CDCl_3$) δ=11.06 (1H, s, CHO), 10.45/44, 9.42, 8.51 (each 1H, s, 5-, 10-, 20-H), 6.52 (1H, q, J=7 Hz, 3-CH), 5.22, 5.07 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.47 (1H, dq, J=2, 7 Hz, 18-H), 4.26 (1H, dt, J=9, 2 Hz, 17-H), 3.87/86 (2H, q, J=8 Hz, 8-CH_2), 3.64/63, 3.59, 3.44 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.50–2.65, 2.20–2.40 (each 2H, m, 17-CH_2CH_2), 2.15 (3H, d, J=7 Hz, 3^1-CH_3), 1.82 (3H, d, J=7 Hz, 18-CH_3), 1.73 (3H, t, J=8 Hz, 8^1-CH_3), 0.33, -1.72 (each 1H, s, NH). MS (FAB) found: *m/z* 580. Calcd for $C_{34}H_{36}N_4O_5$: M^+, 580.

Via hydration of methyl pyropheophorbide-*b* (5d). Similar hydration of **5d** (5.9 mg) by the above procedures gave (3^1R)/(3^1S)=1/1 mixtures of **1** (3.8 mg, 62% yield) after purification by FCC (1.0–1.2% MeOH–CH_2Cl_2).

Via hydration of pyropheophytin-*b*. Similar hydration of pyropheophytin-*b* (9.9 mg) using the above procedures gave (3^1R)/(3^1S)=1/1 mixtures of **1** (3.8 mg, 55% yield) after purification by FCC (1.0–1.2% MeOH–CH_2Cl_2).

Synthesis of methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*b* (2)

Methyl pyropheophorbide-*b* (**5d**, 20 mg) was dissolved in dichloromethane (20 mL), to which neopentyl glycol (22 mg) and *p*-toluene sulfonic acid (40 mg) were added. After 3-h stirring at room temperature, the reaction mixture was poured into pH=8.6 buffer solution prepared by aqueous 0.5 M $NaHCO_3$ (50 mL) and 0.05 M K_2CO_3 (50 mL) solutions. The separated dichloromethane solution was washed with aqueous 4% $NaHCO_3$ solution, washed with brine and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by FCC (5–6% Et_2O–CH_2Cl_2) and recrystallization from CH_2Cl_2 and hexane to give neopentyl-ketal protected compound **5dP** (23 mg, 99%); black solid; mp 112–113°C; VIS (CH_2Cl_2) λ_{max}=660 (rel. 0.30), 605 (0.07), 543 (0.07), 512 (0.11), 419 nm (1.00); ^{1}H NMR ($CDCl_3$) δ=10.10, 9.57, 8.52 (each 1H, s, 5-, 10-, 20-H), 8.01 (1H, dd, J=11, 18 Hz, 3-CH), 6.56 (1H, s, 7-CH), 6.29 (1H, dd, J=1, 18 Hz, 3^1-CH-*trans* to C3^1-H), 6.14 (1H, dd, J=1, 12 Hz, 3^1-CH-*cis* to C3^1-H), 5.26, 5.10 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.48 (1H, dq, J=2, 7 Hz, 18-H), 4.27 (1H, dt, J=9, 2 Hz, 17-H), 4.16, 4.07 (each 2H, d, J=11 Hz, 7^1-OCH_2), 3.86 (2H, q, J=7.5 Hz, 8-CH_2), 3.67, 3.60, 3.39 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.50–2.70, 2.25–2.40 (each 2H, m, 17-CH_2CH_2), 1.85, 0.94 (each 3H, s, 7^4-CH_3), 1.82 (3H, d, J=7 Hz, 18-CH_3), 1.75 (3H, t, J=7.5 Hz, 8^1-CH_3), 0.37, -1.98 (each 1H, s, NH). MS (FAB) found: *m/z* 649. Calcd for $C_{39}H_{45}N_4O_5$: MH^+, 649.

According to procedures similar to those reported by Tamiaki and his colleagues,[30] the above ketal **5dP** (23 mg) was oxidized for 23 h by OsO_4 and $NaIO_4$ to give a mixture (22.7 mg) of the corresponding 3-formyl-chlorin **5gP** [typical δ=11.64 (1H, s, CHO), 10.72, 9.58, 8.81 (each 1H, s, 5-, 10-, 20-H)]: the over-reacted 3,7-diformyl-chlorin **5g** [typical δ=11.67, 11.26 (each 1H, s, CHO), 10.93, 9.76, 8.83 (each 1H, s, 5-, 10-, 20-H)]: unreacted 3-vinyl-chlorin **5dP**=72: 12: 16 determined from the ^{1}H NMR analysis. The crude mixture without FCC purification was used for the following reduction.

Also according to procedures similar to those reported by Tamiaki and his colleagues,[30] the above mixture (22.7 mg) was reduced for 18 h by *t*-$BuNH_2BH_3$ to give a mixture of 3-hydroxymethyl-chlorin **2P**, 3,7-bis(hydroxymethyl)-chlorin **5e** and 3-vinyl-chlorin **5dP**. The crude mixture was easily separated by FCC to give **5dP** (6% Et_2O–CH_2Cl_2), **5e** (2% MeOH–CH_2Cl_2, 2 mg) [selected spectral data, δ=5.74 and 5.61 (each 2H, s, 3-, 7-CH_2) and *m/z* 568 (M^+)], and the desired **2P** (1.2% MeOH–CH_2Cl_2, 13.4 mg; 59% yield for the above two steps); black solid (from CH_2Cl_2–hexane); mp 119°C; VIS (CH_2Cl_2) λ_{max}=655.5 (rel. 0.32), 600 (0.07), 541 (0.07), 509 (0.09), 417 nm (1.00); ^{1}H NMR ($CDCl_3$) δ=10.10, 9.46, 8.51 (each 1H, s, 5-, 10-, 20-H), 6.56 (1H, s, 7-CH), 5.90 (2H, s, 3-CH_2), 5.21, 5.05 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.44 (1H, dq, J=2, 7 Hz, 18-H), 4.23 (1H, dt, J=9, 2 Hz, 17-H), 4.17, 4.07 (each 2H, d, J=11 Hz, 7^1-OCH_2), 3.82 (2H, q, J=7.5 Hz, 8-CH_2), 3.74, 3.60, 3.40 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.50–2.70, 2.25–2.40 (each 2H, m, 17-CH_2CH_2), 1.92, 1.05 (each 3H, s, 7^4-CH_3), 1.79 (3H, d, J=7 Hz, 18-CH_3), 1.71 (3H, t, J=7.5 Hz, 8^1-CH_3), 0.10, -1.87 (each 1H, s, NH). MS (FAB) found: *m/z* 653. Calcd for $C_{38}H_{45}N_4O_6$: MH^+, 653.

3-Hydroxymethyl-7-neopentyl-ketal-protected-formyl-chlorin **2P** (13.4 mg) was dissolved in acetone (20 mL), to which aqueous 1% HCl solution (30 mL) was added. After 15-min stirring at room temperature under N_2, the reaction mixture was poured into ice-water and dichloromethane, washed with aqueous 4% $NaHCO_3$ solution and dried over Na_2SO_4. The solvents were evaporated and the residue was purified with HPLC (retention time was 8.3 min, Cosmosil, MeOH, 1.0 mL/min) to give the titled compound **2** (11.3 mg, 97%); brown solid (from CH_2Cl_2–hexane); mp 115–117°C; VIS (CH_2Cl_2) λ_{max}=649.5 (rel. 0.20), 595.5 (0.06), 526 (0.07), 435.5 (1.00), 415 nm (0.49); ^{1}H NMR ($CDCl_3$) δ=10.82 (1H, s, CHO), 10.14, 9.07, 8.52 (each 1H, s, 5-, 10-, 20-H), 5.86 (2H, s, 3-CH_2), 5.21, 5.05 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.47 (1H, dq, J=2, 7 Hz, 18-H), 4.25 (1H, dt, J=9, 2 Hz, 17-H), 3.52 (2H, q, J=7.5 Hz, 8-CH_2), 3.65, 3.48, 3.40 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.55–2.70, 2.25–2.40 (each 2H, m, 17-CH_2CH_2), 1.84 (3H, d, J=7 Hz, 18-CH_3), 1.57 (3H, t, J=7.5 Hz, 8^1-CH_3), 0.07, -1.84 (each 1H, s, NH). MS (FAB) found: *m/z* 567. Calcd for $C_{33}H_{35}N_4O_5$: MH^+, 567.

Transformation of zinc 7-formylchlorin to zinc 7-methylchlorin

To a methanol solution (9 mL) of zinc methyl bacteriopheophorbide-*f* (**1**, ca. 20 μg) was added $NaBH_3CN$ (4 mg) at room temperature with stirring under N_2. One molar methanol solution of HCl was added dropwise to the

mixture, which changed the Q_y-peak from 635 to 647 nm. After the complete shift in the visible peak, ethyl acetate and aqueous 4% $NaHCO_3$ solution was added to the reaction mixture and the upper organic phase was separated. The aqueous phase was re-extracted with ethyl acetate until the solution was decolorized. The combined organic phase was dried over Na_2SO_4. The solvent was evaporated and the residue was zinc-metallated[30] to give zinc methyl bacteriopheophorbide-*d* (**3**) after purification of HPLC (retention time=39 (3^1R) and 43 min (3^1S), Cosmosil, MeOH/H_2O=4/1, 1.0 mL/min).

Spectral data

Zinc methyl bacteriopheophorbide-*f* ($3^1R/3^1S$=1/1) (Zn-1). 91%; retention time was 8.1 min (Gelpack, MeOH, 1.0 mL/min); light brown solid (precipitated from hexane); mp >300°C; VIS (THF) λ_{max}=629 (rel. 0.34), 581 (0.07), 448 nm (1.00); ^{1}H NMR ($CDCl_3$ — 0.2% C_5D_5N) δ=11.22 (1H, s, CHO), 10.18/13, 9.59, 8.18 (each 1H, s, 5-, 10-, 20-H), 6.41/37 (1H, q, *J*=6.5 Hz, 3-CH), 5.09, 4.96 (1H, d, *J*=20 Hz, 13^1-CH), 4.32 (1H, dq, *J*=2, 7 Hz, 18-H), 4.11 (1H, dt, *J*=8, 2 Hz, 17-H), 4.07 (2H, q, *J*=7 Hz, 8-CH_2), 3.62, 3.59, 3.32/30 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.20–2.50 (4H, m, 17-CH_2CH_2), 2.12/11 (3H, d, *J*=6.5 Hz, 3^1-CH_3), 1.80 (3H, d, *J*=7 Hz, 18-CH_3), 1.70 (3H, t, *J*=7 Hz, 8^1-CH_3). MS (FAB) found: *m/z* 642. Calcd for $C_{34}H_{34}N_4O_5{}^{64}Zn$: M^+, 642.

Zinc methyl 3^1R-bacteriopheophorbide-*f* (Zn-1R). Retention time was 130–133 min (Cosmosil, MeOH/H_2O=2/1, 1.5 mL/min); light brown solid (precipitated from hexane); VIS (THF) λ_{max}=628 (rel. 0.34), 580 (0.07), 448 nm (1.00); ^{1}H NMR ($CDCl_3$ — 0.2% C_5D_5N) δ=11.22 (1H, s, CHO), 10.13, 9.59, 8.17 (each 1H, s, 5-, 10-, 20-H), 6.41 (1H, q, *J*=7 Hz, 3-CH), 5.08, 4.94 (1H, d, *J*=20 Hz, 13^1-CH), 4.28 (1H, dq, *J*=2, 7.5 Hz, 18-H), 4.09 (1H, dt, *J*=8, 2 Hz, 17-H), 4.00 (2H, q, *J*=7 Hz, 8-CH_2), 3.61, 3.57, 3.31 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.20–2.50 (4H, m, 17-CH_2CH_2), 2.09 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.79 (3H, d, *J*=7.5 Hz, 18-CH_3), 1.69 (3H, t, *J*=7 Hz, 8^1-CH_3).

Zinc methyl 3^1S-bacteriopheophorbide-*f* (Zn-1S). Retention time was 137–140 min (Cosmosil, MeOH/H_2O=2/1, 1.5 mL/min); light brown solid (precipitated from hexane); VIS (THF) λ_{max}=628 (rel. 0.34), 580 (0.07), 448 nm (1.00); ^{1}H NMR ($CDCl_3$ — 0.2% C_5D_5N) δ=11.22 (1H, s, CHO), 10.18, 9.58, 8.16 (each 1H, s, 5-, 10-, 20-H), 6.37 (1H, q, *J*=7 Hz, 3-CH), 5.08, 4.94 (1H, d, *J*=20 Hz, 13^1-CH), 4.29 (1H, dq, *J*=2, 7.5 Hz, 18-H), 4.08 (1H, dt, *J*=8, 2 Hz, 17-H), 4.03 (2H, q, *J*=7 Hz, 8-CH_2), 3.61, 3.58, 3.28 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.20–2.50 (4H, m, 17-CH_2CH_2), 2.11 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.78 (3H, d, *J*=7.5 Hz, 18-CH_3), 1.67 (3H, t, *J*=7 Hz, 8^1-CH_3).

Zinc methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*b*(zinc methyl 3^1-demthyl-bacteriopheophorbide-*f*, Zn-2). 90%; retention time was 6.9 min (Gelpack, MeOH, 1.2 mL/min) or 24 min (Cosmosil, MeOH/H_2O=2/1, 1.5 mL/min); light brown solid (precipitated from hexane); mp >300°C; VIS (THF) λ_{max}=630 (rel. 0.32), 583 (0.06), 449 nm (1.00); ^{1}H NMR ($CDCl_3$ — 0.2% C_5D_5N) δ=11.20 (1H, s, CHO), 10.01, 9.60, 8.22 (each 1H, s, 5-, 10-, 20-H), 5.79 (2H, s, 3-CH_2), 5.10, 4.97 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.29 (1H, dq, *J*=2, 7 Hz, 18-H), 4.11 (1H, dt, *J*=9, 2 Hz, 17-H), 4.09 (2H, q, *J*=7.5 Hz, 8-CH_2), 3.63, 3.59, 3.28 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.10–2.60 (4H, m, 17-CH_2CH_2), 1.79 (3H, t, *J*=7.5 Hz, 8^1-CH_3), 1.69 (3H, d, *J*=7 Hz, 18-CH_3). MS (FAB) found: *m/z* 628. Calcd for $C_{33}H_{32}N_4O_5{}^{64}Zn$: M^+, 628.

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