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Effect of C7-Substituent on Self-Assembly of Chlorosomal Chlorophylls

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Bacteriochlorophyll *d* analogues that possessed a series of bulky substituents of OMe, OCOMe, and OCO^{t}Bu at the C7^1-position were synthesized (Zn-complexes of methyl 7^1-substituted-3-(1-hydroxy-methyl)pyropheophorbide *a*). Aggregation behavior of these pigments was examined in comparison with a natural-type compound (C7^1-H) to elucidate the effects of size of the C7^1-moiety on self-assembly of the chlorins. These C7^1-substituted Zn-chlorins formed self-aggregates in 1% THF/hexane. The aggregates gave almost the same visible absorption bands, especially a *ca.* 2000-cm^{-1} red-shifted Q_y peak; the spectra were essentially similar to those of the natural-type Zn-chlorin (C7^1-H). All the aggregates of the C7^1-substituted compounds showed similar exciton-type CD couplets in the red-shifted Q_y region, which were different from the feature of the couplet of the C7^1-H Zn-chlorin aggregate. These spectral data suggested that the C7^1-moiety did not interrupt the intermolecular linkages between chlorin molecules but altered the supramolecular structure which influences the long-range chirality.

Keywords: Aggregate, Chlorophyll, Chlorosome, Self-assembly, Supramolecule

Abbreviations: BChl, bacteriochlorophyll, CD, circular dichroism, Chl, chlorophyll, DMAP, 4-(*N*,*N*-dimethylamino)pyridine, EDC, 3-ethyl-1-(3-dimethylaminopropyl)carbodiimide, Et_3N, triethylamine, FCC, flash column chromatography, THF, tetrahydrofuran

INTRODUCTION

Functional cores of chlorosomes, extramembranous antennae of green photosynthetic bacteria, are cylindrical self-aggregates of C3^1-OH-type chlorophyll (Chl) derivatives (*e.g.* bacteriochlorophyll (BChl) *d*, **1**, Fig. 1). Chlorosomal Chls have some homologues with variations in the peripheral substituents at the C7, C8, C12, C17 and C20-positions [1]. Although such molecular diversity may influence the supramolecular structures of the self-assemblies of these chlorosomal-type Chls, neither the exact molecular arrangements of the *in vivo* Chl self-aggregates nor the effects of these peripheral substituents on the molecular arrangements have yet been determined.

It has been reported that sizes of the C8, C12, C13^2, C17 and C20-substituents of C3^1-OH-type Chls influence *in vitro* aggregation behaviors [2–10]. Self-aggregates of BChl possessing more bulky C8-moiety give more red-shifted Q_y peaks. Smith and his colleagues found that natural chlorosomes containing BChl *c* homologues

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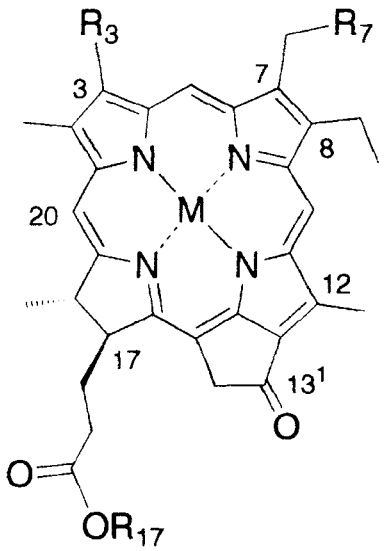


	R_3	R_7	R_{17}	M
1	CH(Me)OH	H	Stearyl etc.	Mg
2	CH_2OH	OMe	Me	Zn
3	CH_2OH	OCOMe	Me	Zn
4	CH_2OH	OCO^tBu	Me	Zn
5	CH_2OH	H	Me	Zn
6	$CH{=}CH_2$	OH	Me	H_2
7	$CH{=}CH_2$	H	Me	H_2
8	CH_2OH	H	3-methyl-1-butyl	Zn

FIGURE 1 Molecular structure of metallochlorins with partial carbon numbering according to the IUPAC system

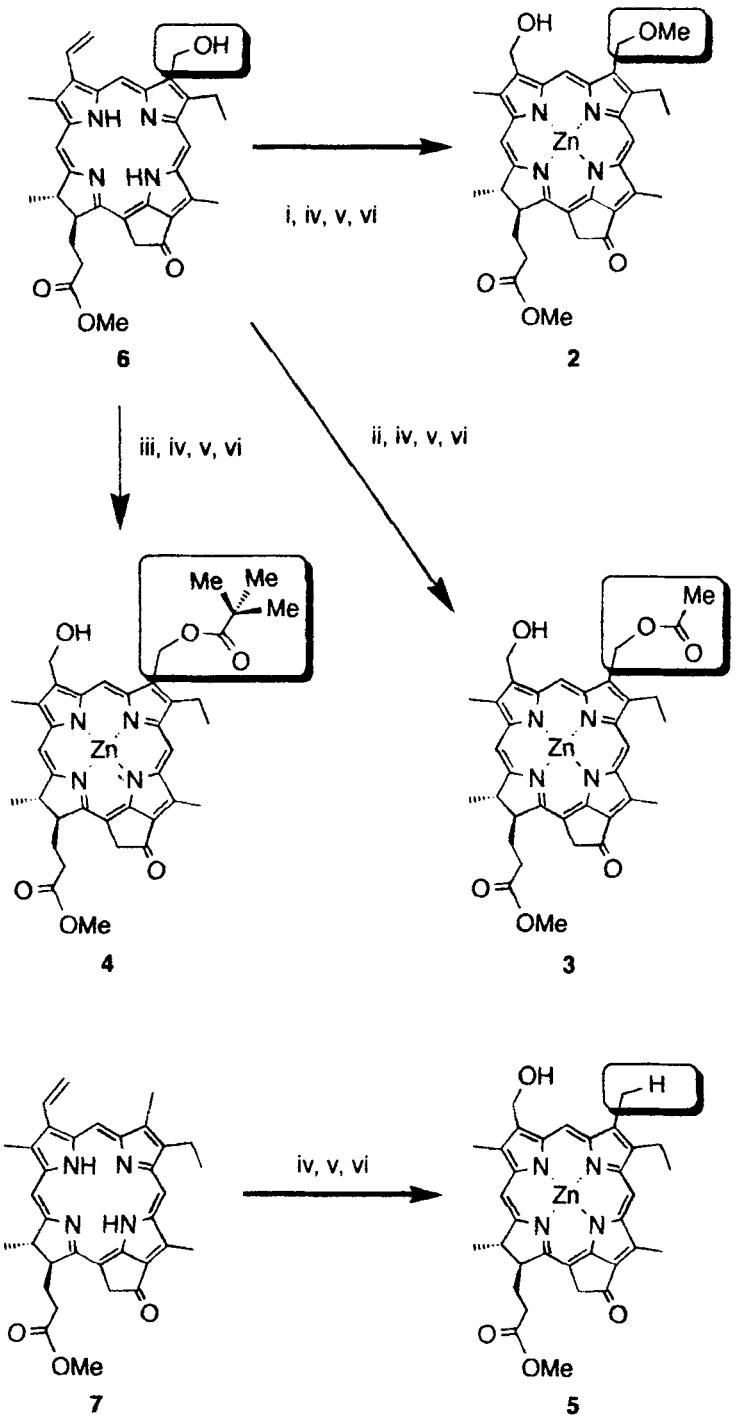
with more bulky C8-moiety (comparing a mixture of Et- and ^{n}Pr -derivatives with a mixture of Et-, ^{n}Pr-, ^{i}Bu- and ^{t}Bu-CH_2-ones) gave a more red-shifted Q_y absorption band [2, 3]. Nozawa *et al.* examined aggregations of isolated BChl *c* homologues in 2% dichloromethane/hexane and showed that the Q_y absorption maximum of the aggregate solution shifted from 717 ~ 719 nm to 721 ~ 725 nm in going from C8-Et to ^{n}Pr, and then to ^{i}Bu [4]. Uehara *et al.* also reported that the Q_y band of the BChl *c* self-aggregate formed in an aqueous lipid solution shifted from 724 to 745 nm in going from C8-Et to ^{n}Pr, and to ^{i}Bu [5]. Although it is well known that the $Q_y$peak of the chlorosome composed of BChl *e* (C7-CHO) was more blue-shifted than that of BChl *c* (C7-Me), to our knowledge no reports are available on the effects of size of the C7-moiety on the aggregation behavior of chlorosomal Chls.

We synthesized several $C3^1$-OH-type zinc-chlorins **2** ~ **5** (Fig. 1) which had a series of substituents (H, OMe, OCOMe, and OCO^tBu) at the $C7^1$-position, and examined their aggregation behaviors in nonpolar organic solvent (1% THF/hexane). The synthetic Zn-chlorins gave similar absorption spectra in the aggregated states irrespective of the $C7^1$-substituent, but the aggregate CD spectra showed differences in the Q_y region. The $C7^1$-moiety should not interrupt the self-assembly of the chlorins but affect the supramolecular structure of the aggregate.

RESULTS AND DISCUSSION

Synthesis of C7-substituted Zn-chlorins 2 ~ 4

We synthesized $C3^1$-OH-type Zn-Chls which possessed the OMe (**2**), OCOMe (**3**), or OCO^tBu (**4**) group at the $C7^1$-position (Scheme 1). The $C7^1$-OH group of methyl 7-(1-hydroxymethyl)pyropheophorbide *a* (**6**), prepared as described [11], was subjected to etherification or esterification (i ~ iii steps in Scheme 1). The $C7^1$-OMe compound was obtained by 24-h stirring of **6** in MeOH with concentrated H_2SO_4 (10% vol/vol). Acetylation of the $C7^1$-OH group was done by treatment of **6** with acetic anhydride in pyridine. The $C7^1$-OH group of **6** was also esterified with pivalic acid in dry CH_2Cl_2 using 3-ethyl-1-(3-dimethylaminopropyl)carbodiimide (EDC), 4-(*N*,*N*-dimethylamino)pyridine (DMAP), and triethylamine (Et_3N). These substitutions were confirmed by FAB-MS ($C7^1$-OMe, 578 (M^+); $C7^1$-OCOMe, 606 (M^+); $C7^1$-OCO^tBu, 649 (MH^+)) and the proton resonance signals of the additional methyl groups.



SCHEME 1 Synthesis of Zn-chlorins **2** ~ **5** (all the reactions were done at room temperature under nitrogen in the dark). (i) conc. H_2SO_4 / MeOH, 24 h; (ii) Ac_2O / pyridine, 20 h; (iii) ^{t}BuCOOH, EDC, DMAP, Et_3N / dry CH_2Cl_2, 12 h; (iv) OsO_4, $NaIO_4$ / aq. AcOH, THF, 20 h; (v) ^{t}BuNH$_2$BH$_3$ / CH_2Cl_2, 20 h; (vi) $Zn(OAc)_2$ / MeOH, 2 h

Thus obtained $C7^1$-substituted methyl pyropheophorbides *a* (C3-vinyl) were converted to the desired compounds **2** ~ **4** (C3-CH_2OH) according to preparation procedures of the reference compound **5** ($C7^1$-H, natural-type zinc chlorin) as described (iv ~ vi steps in Scheme 1, and ref. 12): oxidation of the C3-vinyl group with catalytic OsO_4 and equivalent $NaIO_4$ to afford the C3-formyl compounds, reduction of the formed formyl moiety with *tert*-butylamine-borane complex, and, finally, zinc-metallation. The reactions on the C3-position were successful even for the sterically bulky $C7^1$-OCO^{t}Bu derivative.

Visible absorption spectra

Figure 2a shows the visible absorption spectra of monomeric **2** ~ **5** in THF. $C7^1$-Substituted compounds **2** ~ **4** gave essentially identical spectral features to $C7^1$-unsubstituted **5**. The Q_y absorption bands of monomeric **2** ~ **4** peaked at nearly the same wavelength positions of 639 ~ 640 nm with the band widths of 330 ~ 350 cm^{-1} (full widths at half maxima). All the Soret absorption maxima located at 426 ~ 427 nm. Fluorescence emission spectra of **2** ~ **4** excited at the Soret maximum were also almost the same, and the main peak was at 642 ~ 643 nm (Stokes shift, *ca.* 70 cm^{-1}). The Q_y absorption peak of monomeric **5** located at 647 nm (band width, 320 cm^{-1}).

Dilution of the monomeric solution of **2** ~ **4** with hexane induced an immediate red-shift of the Q_y absorption band to 729 ~ 732 nm, indicating the formation of self-aggregates (Fig. 2b). The aggregate absorption spectra of compounds **2** ~ **4** were closely similar to each other. The Q_y bands experienced nearly the same extent of red-shift (*ca.* 2000 cm^{-1}) through the aggregation. The spectral features of the aggregate Q_y bands were almost common, tailing off at the shorter wavelength region and falling steeply at the longer-wavelength side. The Soret maxima located at *ca.* 460 nm, and were less intense than the Q_y bands. Few molecules were left in the monomeric state, which is demonstrated by a less pronounced monomer absorption peak at around 640 nm. Such characteristics are almost the same as those of the aggregate of the $C7^1$-H compound **5** (red-shift = 1930 cm^{-1}; ref. 12). Near coincidence of the red-shift values proves that the strength of intermolecular interactions is nearly the same between the component molecules in the aggregates of **2** ~ **5**. Therefore, a dominant aggregate species of **2** ~ **5** possessed an essentially similar array of the Q_y transition moments, a J-aggregate type one as postulated for **5** [12–16]. This leads to the intermolecular interactions between the component molecules in these aggregates being common irrespective of the $C7^1$-substituent [17]: coordination interaction of Zn ··· O(H)-$C3^1$ and a hydrogen-bonding of $C3^1$-OH ··· O=$C13^1$, as the *in vitro* **5** aggregate and the *in vivo* self-aggregates do [12, 16].

CD spectra

The monomeric Zn-chlorins **2** ~ **4** in THF showed a weak, negative CD component at *ca.* 640 nm, corresponding to the Q_y transition, and a positive Soret CD signal at around 430 nm (spectra not shown). The spectral characteristics were essentially the same as those of compound **5** [12]. In the aggregated states in 1% THF/hexane, **2** ~ **4** gave mutually quite similar exciton-type CD couplets in the Q_y region (Fig. 2c). The positive CD component was situated at longer wavelength position than the paired negative signal, and was more intense than the negative one. In addition, the positive CD lobe peaked at longer wavelength than or at one equal to the Q_y absorption peak (Fig. 2b). In contrast, the spectral feature of the CD couplet of the **5** aggregate (broken curve in Fig. 2c) was different from those of the **2** ~ **4** aggregates. A new negative CD lobe was observed at 748 nm, in addition to an intense positive component at 732 nm and a small negative one at 701 nm. The 748-nm negative CD lobe was more intense and sharper than the other negative one.

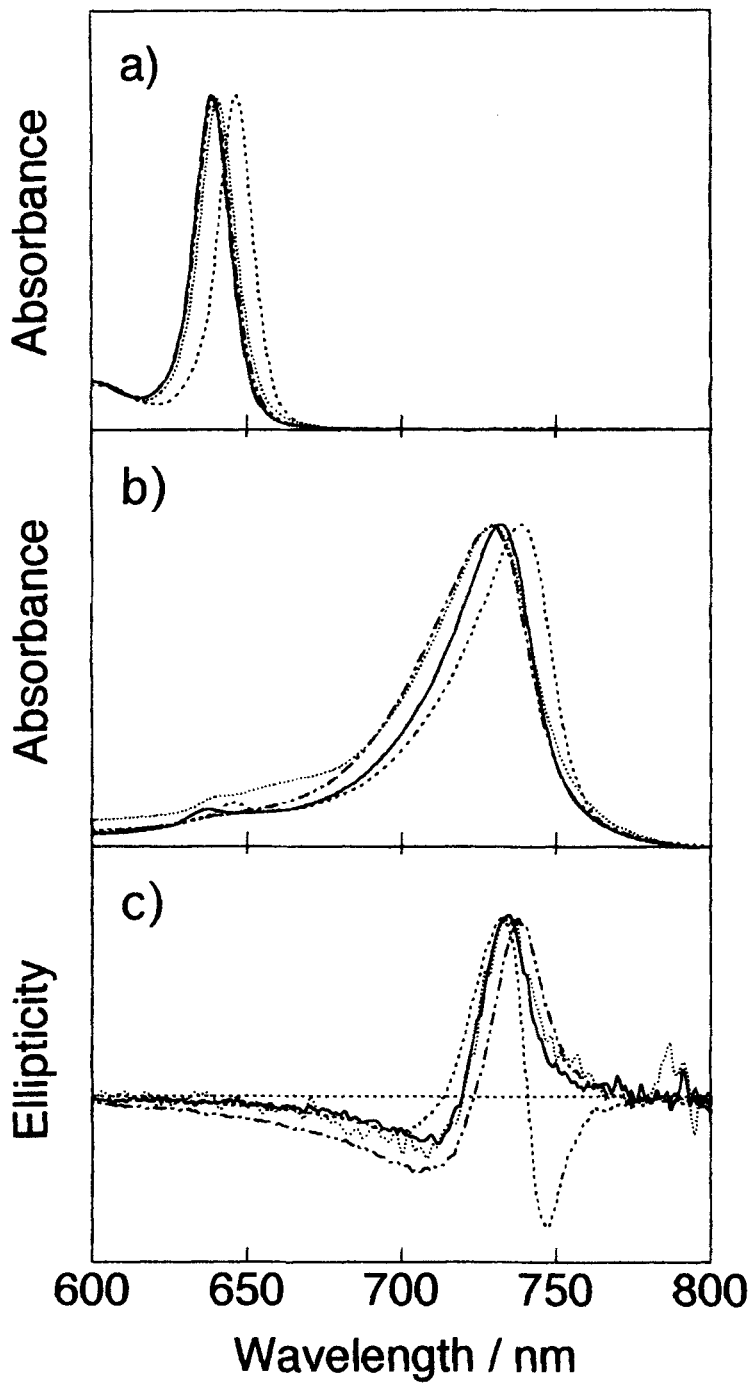


FIGURE 2 (a) Visible absorption spectra of $C7^1$-substituted Zn-chlorins in the monomeric state (*ca.* 20 μM, in THF, at room temperature); (b) visible absorption spectra of $C7^1$-substituted Zn-chlorins in the aggregated state (*ca.* 10 μM, in 1% THF/hexane, at room temperature); (c) CD spectra of $C7^1$-substituted Zn-chlorins in the aggregated state (*ca.* 10 μM, in 1% THF/hexane, at room temperature). All the spectra were normalized at the positive peak. $C7^1$-OMe (**2**): ·····; $C7^1$-OCOMe (**3**): ·-·-; $C7^1$-OCO^{t}Bu (**4**): –; $C7^1$-H (**5**): - - -

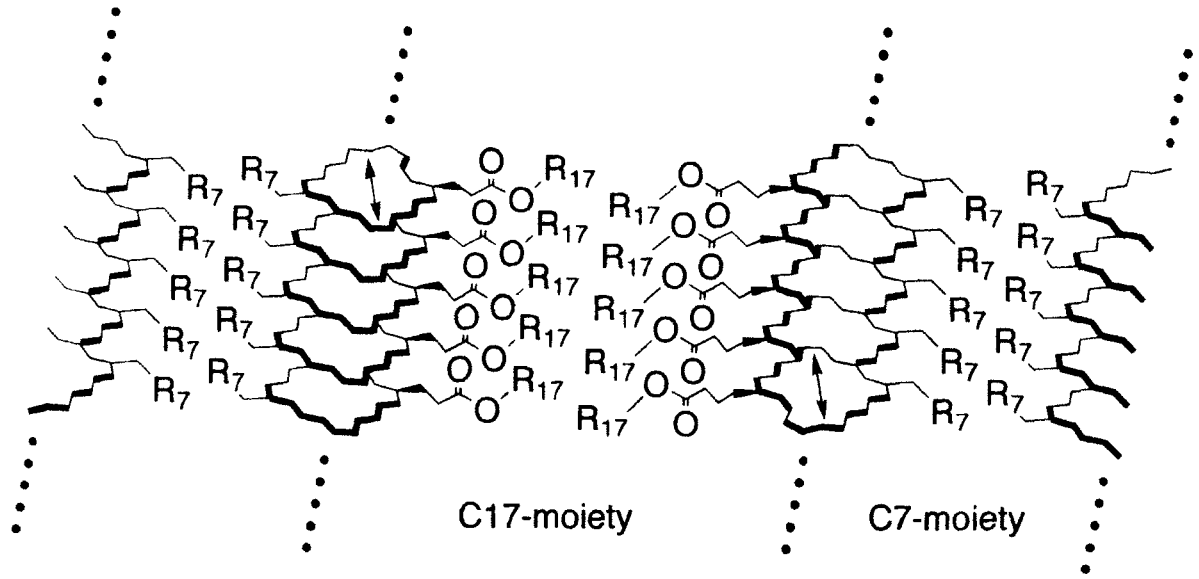


FIGURE 3 Schematic representation of a possible structural motif of the self-aggregate of the C7-substituted Zn-chlorins. Each chlorin molecule links together with neighboring molecules by the intermolecular interactions of $C3^1$-OH ···Zn and $C3^1$-OH ··· O=$C13^1$, and forms a linear molecular array. The arrays associate to form a layered structure. An arrow in the chlorin macrocycle represents the Q_y transition moment

The differences in the CD spectra reflect differences in the supramolecular structures of the aggregates. The broad Q_y absorption bands of the chlorophyllous J-aggregates can be interpreted as a widely spread exciton manifold [14, 15]. Relative intensities and polarities of CD components induced by these exciton levels determine the overall CD spectrum [21, 22]. The theory predicted that a linear molecular array (J-aggregate) of 20 ~ 40 Chl molecules is sufficient to show the maximum value for red-shift of absorption bands [14, 23, 24], while CD signals depend not only on interactions between neighboring Chl molecules but on the supramolecular architecture that can affect the long-range chirality [25, 26]. In this context, nearly the same red-shifts observed for the **2** ~ **5** aggregates demonstrate that compounds **2** ~ **5** can form essentially the same linear molecular arrays (J-aggregates) with sufficiently large aggregation numbers, while the differences in the CD spectra, especially between **5** and **2** ~ **4**, suggest that further association of such J-aggregates, which gives the supramolecular architecture, may occur in different manners due to steric hindrance of the C7-substituent.

Effect of the C7-moiety on the supramolecular structure of the self-aggregates

We earlier synthesized Zn-complexes of $C3^1$-OH-type chlorins possessing a variety of C17-alkyl chains [7]. The C17-(3-methyl-1-butyl) propionate derivative (**8**, Fig. 1) gave a J-aggregate type absorption spectrum with its Q_y maximum at 734 nm in 1% THF/hexane. The CD spectrum of this aggregate solution showed a dispersed-type feature in the Q_y region. The positive CD lobe at *ca.* 733 nm was sharper and more intense than the negative one at around 705 nm. It is noted that such spectral characteristics closely resemble those of the aggregates of **2** ~ **4**.

We had found that the self-aggregates of the series of C17-substituted chlorins including **8** changed the Q_y CD spectral feature systematically depending on the chain-length of the C17-substituent, in spite of the nearly identical visible absorption spectra (in 1% THF/hexane) [7]. When Chl molecules link together with the intermolecular interactions of Zn ···($C3^1$)OH ··· O=$C13^1$, the C7- and C17-moieties protrude to the peripheries of the J-aggregate (Fig. 3). Such J-aggregates may associate to form a layered motif that can extend into a supramolecular architecture like micelles or liposomes (Fig. 3), as surfactants and lipids do [13, 29–31]. Length and bulkiness of the C7- and C17-substituents can sterically determine spacing between the layers as well as curvature (or pitch) that the layers form [32], which may influence the CD spectrum of the aggregate [25, 26]. Though the exact supramolecular structures of the self-aggregates are not yet identified, we tentatively assume that the observed similarities in the CD spectral feature of the aggregates of **2** ~ **4** (and **8**) reflected similarities in the spacing and the curvature that the C7- (and C17-) moiety provides [33], in comparison with presumably the most compactly packed structure of the aggregate of the natural-type compound **5** when closely interacting. Minor differences among the CD spectra of the aggregates of **2** ~ **4** may also reflect the steric hindrance and the packing effect of the C7-moiety.

In summary, the size of the C7-substituent did not affect the linearity of the array of the Q_y transition moments in the self-aggregates but can alter the structure of the supramolecule. The effect would be comparable to that of the C17-long alkyl chain.

EXPERIMENTAL

Apparatus and materials

Synthetic zinc chlorins **2** ~ **4** were purified either by flash column chromatography (FCC; Merck Kieselgel 60) or by a reversed-phase HPLC (ODS column: GL-OP100, 6.0 mmϕ × 150 mm (Hitachi Chemical Co., Ltd.), room temperature; detected at 427 ~ 429 nm). All synthetic compounds were characterized by ^{1}H-NMR and mass spectra. Proton NMR spectra were recorded on a 300 MHz NMR spectrometer (Bruker AC-300) in $CDCl_3$ (for free bases) or $CDCl_3$ containing 10 vol% CD_3OD (for Zn-chlorins) solutions operating at 20 °C. Resonance peaks were assigned on the basis of results of 2D-NMR and data of related compounds [11, 12, 34]. Mass spectra were obtained using a fast atomic bombardment mass spectrometer JEOL HX-110 (FAB matrix, 3-nitrobenzyl alcohol). Visible absorption, CD and fluorescence spectra were recorded at room temperature on a Hitachi spectrophotometer U-3500, a JASCO spectropolarimeter J-720W, and a Hitachi fluorescence spectrophotometer F-3500, respectively.

Reagent-grade hexane, THF, methanol, pyridine and dichloromethane were purchased from Nacalai Tesque (Kyoto, Japan). Hexane and THF were dried over CaH_2 and distilled prior to spectroscopic measurements.

Methyl 7-(1-hydroxymethyl)pyropheophorbide *a* (**6**) was prepared from Chl *b* according to the prescribed method [11].

Synthesis of methyl 7-(1-methoxymethyl)-pyropheophorbide *a* ($C7^1$-OMe)

Concentrated sulfuric acid (1 mL) was added dropwise to an ice-chilled methanol solution (10 mL) of **6** (*ca.* 11 μmol) and stirred for 24 h at room temperature. The solution was poured into ice water and extracted with CH_2Cl_2. The organic phase was neutralized with aq. $NaHCO_3$, and washed with brine. The solution was dried over Na_2SO_4, and evaporated to dryness. The residue was purified by FCC (eluted with CH_2Cl_2/Et_2O = 100/6). Visible (CH_2Cl_2): λ_{max} (relative intensity) 663 (0.38); 606 (0.07); 543 (0.07); 513 (0.10); 419 nm (1.00). ^{1}H-NMR ($CDCl_3$): δ 9.61 (s, CH-5); 9.56 (s, CH-10); 8.55 (s, CH-20);

7.99 (dd, J = 12, 18 Hz, CH-3^1); 6.32 (dd, J = 1, 18 Hz, CH-3^2-*cis*); 6.18 (dd, J = 1, 12 Hz, CH-3^2-*trans*); 5.53 (s, CH_2-7^1); 5.27, 5.11 (2d, J = 20 Hz, CH_2-13^2); 4.49 (dq, J = 2, 7 Hz, CH-18); 4.29 (dt, J = 2, 8 Hz, CH-17); 3.78 (q, J = 8 Hz, CH_2-8^1); 3.68, 3.67, 3.62 (3s, CH_3-7^3 + 12^1 + 17^5); 3.40 (s, CH_3-2^1); 2.50 ~ 2.77, 2.22 ~ 2.38 (2m, CH_2-17^1 + 17^2); 1.82 (d, J = 7 Hz, CH_3-18^1); 1.75 (t, J = 8 Hz, CH_3-8^2); −1.66 (s, NH) [35]. MS (FAB): *m/z* 578 (M^+).

Synthesis of methyl 7-(1-acetoxymetyl)-pyropheophorbide *a* (C7^1-OAc)

Compound **6** (*ca.* 1.4 μmol) was dissolved in pyridine, and cooled in an ice-water bath. To this solution was added acetic anhydride (2 eq., 0.3 mL) dropwise. The solution was stirred for *ca.* 20 h under nitrogen at room temperature. The reaction mixture was poured into ice water and extracted with CH_2Cl_2. The organic phase was washed with aq. HCl, aq. $NaHCO_3$, and brine. Then, the solution was dried over Na_2SO_4, and evaporated to dryness. The residue was purified by FCC (eluted with CH_2Cl_2/Et_2O = 100/4 ~ 100/10). Visible (CH_2Cl_2): λ_{max} 661 (0.31); 605 (0.06); 543 (0.05); 513 (0.09); 421 nm (1.00). ^{1}H-NMR ($CDCl_3$): δ 9.57 (s, CH-5); 9.51 (s, CH-10); 8.58 (s, CH-20); 8.00 (dd, J = 12, 18 Hz, CH-3^1); 6.32 (dd, J = 1, 18 Hz, CH-3^2-*cis*); 6.20 (dd, J = 1, 12 Hz, CH-3^2-*trans*); 6.21 (s, CH_2-7^1); 5.27, 5.11 (2d, J = 20 Hz, CH_2-13^2); 4.50 (dq, J = 2, 7 Hz, CH-18); 4.30 (dt, J = 2, 8 Hz, CH-17); 3.81 (q, J = 8 Hz, CH_2-8^1); 3.67 (s, CH_3-12^1); 3.62 (s, CH_3-17^5); 3.41 (s, CH_3-2^1); 2.5 ~ 2.8, 2.2 ~ 2.4 (2m, CH_2-17^1 + 17^2); 2.20 (s, CH_3-7^4); 1.83 (d, J = 7 Hz, CH_3-18^1); 1.75 (t, J = 8 Hz, CH_3-8^2); 0.39, −1.73 (2s, NH). MS (FAB): *m/z* 606 (M^+).

Synthesis of methyl 7-(1-pivaloyloxymethyl)-pyropheophorbide *a* (C7^1-OCO^{t}Bu)

Compound **6** (*ca.* 3 μmol) was dissolved in freshly dried CH_2Cl_2, and the solution was chilled in an ice water bath. Pivalic acid (6 eq.), DMAP (6 eq.), Et_3N (6 eq.), and EDC (6 eq.) were added to this cold solution, and stirred for *ca.* 12 h under nitrogen at room temperature. The reaction mixture was washed with 3 vol% aq. HCl, sat. aq. Na_2CO_3, and brine. The organic phase was dried over Na_2SO_4, and evaporated to dryness. Pivaloyl ester was isolated using FCC (eluted with CH_2Cl_2/Et_2O = 100/5 ~ 100/10). Visible (CH_2Cl_2): λ_{max} 661 (0.33); 605 (0.06); 543 (0.05); 512 (0.09); 420 nm (1.00). ^{1}H-NMR ($CDCl_3$): δ 9.60 (s, CH-10), 9.49 (s, CH-5); 8.57 (s, CH-20); 7.99 (dd, J = 12, 18 Hz, CH-3^1); 6.30 (dd, J = 1, 18 Hz, CH-3^2-*cis*); 6.18 (dd, J = 1, 12 Hz, CH-3^2-*trans*); 6.21 (s, CH_2-7^1); 5.27, 5.11 (2d, J = 20 Hz, CH_2-13^2); 4.49 (dq, J = 2, 7 Hz, CH-18); 4.30 (dt, J = 2, 8 Hz, CH-17); 3.83 (q, J = 8 Hz, CH_2-8^1); 3.68 (s, CH_3-12^1); 3.61 (s, CH_3-17^5); 3.41 (s, CH_3-2^1); 2.1 ~ 2.4, 2.4 ~ 2.8 (2m, CH_2-17^1 + 17^2); 1.81 (d, J = 7 Hz, CH_3-18^1); 1.75 (t, J = 8 Hz, CH_3-8^2); 1.25 (s, $(CH_3)_3$-7^5); − 1.69 (s, NH) [35]. MS (FAB): *m/z* 649 (MH^+).

Conversion of methyl C7^1-substituted-pyropheophorbides *a* to Zn-chlorins **2** ~ **4**

Compounds **2** ~ **4** were obtained following procedures similar to the synthesis of **5** (C7^1-H, natural-type zinc chlorin) as described previously (iv − vi steps in Scheme 1; ref. 12). The methods and data are briefly described below. (iv) The C3-vinyl group of the methyl C7-substituted-pyropheophorbides *a* was converted to formyl group with $NaIO_4$ and catalytic OsO_4 in THF (20 h, at room temperature). The formation of formyl moiety was confirmed by disappearance of the C3-vinyl proton resonance peaks (6 ~ 8 ppm) and concomitant appearance of the formyl proton signal at 11.6 ppm as well as by red-shift of the absorption peaks (*ex.* Q_y bands, 661 ~ 663 nm → 687 ~ 689 nm in CH_2Cl_2). (v) The C3-formyl groups formed were reduced by *t*-butylamine-borane complex (1/1) in CH_2Cl_2 (20 h, at room temperature) to yield methyl

7-substituted-3-(1-hydroxymethyl)pyropheophorbides *a* (free base derivatives of **2** ~ **4**). The reduction gave a C3-methylene proton resonance peak at around 5.92 ppm at the expense of the formyl proton signal, and a blue-shift of the absorption bands (*ex.* Q_y bands, 687 ~ 689 nm → *ca.* 656 nm in CH_2Cl_2). (vi) Reaction of thus obtained methyl 7^1-substituted-3-(1-hydroxymethyl)pyropheophorbides *a* with zinc acetate in methanol (2 h, at room temperature) followed by isolation using the reversed-phase HPLC gave pure **2** ~ **4**. The reaction was confirmed by ^{1}H-NMR, FAB-MS, and visible absorption spectroscopy, especially by blue-shifts of the Q_y absorption bands of **2** ~ **4** in comparison with the corresponding free bases, which is a clear marker of zinc metallation of chlorins: **2** (THF): λ_{max} 640 (0.51); 597 (0.09); 426 (1.00); 405 nm (0.50); **3** (THF): λ_{max} 639 (0.52); 595 (0.08); 427 (1.00); 405 nm (0.47); **4** (THF): λ_{max} 639 (0.52); 594 (0.08); 427 (1.00); 405 nm (0.45).

Preparation of aggregates

Zn-chlorin was dissolved in dry THF (50 μL). The solution was diluted quickly with dry hexane (5 mL; final concentration, nearly 10 μM on the monomer basis) and shaken rigorously, resulting in the immediate formation of aggregates. Several milliliters of the aggregate solution was put into a cuvette and subjected to spectroscopic measurements.

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