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Self-aggregation of Synthetic Protobacteriochlorophyll-*d* Derivatives¶

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ABSTRACT

3^1-Racemically pure zinc 3^1-hydroxy-13^1-oxo-porphyrins (zinc methyl 17,18-dehydro-bacteriopheophorbides-*d*) as well as their 3^1-demethyl form were prepared by modifying chlorophyll-*a* through oxidation by 2,3-dichloro-5,6-dicyano-benzoquinone. From visible, circular dichroism and infrared spectral analyses, these synthetic pigments self-aggregated in 1%(vol/vol) tetrahydrofuran and cyclohexane to give large oligomers by an intermolecular bonding of 13-C=O···H–O(3^1)···Zn(central) and π–π interaction of the porphyrin chromophores. The supramolecular structures are similar to those of the corresponding chlorins and a core part of extramembranous light-harvesting antennas of photosynthetic green bacteria. The 17,18-dehydrogenation of a chlorin to porphyrin moiety did not disturb its self-aggregation, and the synthetic zinc porphyrins are good models for naturally occurring, self-aggregative bacteriochlorophylls.

INTRODUCTION

Self-aggregation of porphyrins and chlorophylls (Chls) has attracted much attention in the investigation of natural photosynthetic systems as well as in the construction of artificial photoactive devices. Two self-aggregates of porphyrinoids (cyclic tetrapyrroles) without any assistance of peptide scaffolds are naturally observed: (I) self-aggregates of Fe(III)-protoporphyrin-IX in malaria haemozoin (1) and (II) self-aggregates of bacteriochlorophyll(BChl)*s*-*c*/*d*/*e* in extramembranous light-harvesting antenna of photosynthetic green bacteria (2–6). The latter systems are called ''chlorosomes'' because of their green colored, submicrometer-sized apparatus (green + body = chloro + some). In a chlorosome, a large quantity of BChl-*c*/*d*/*e* molecules self-aggregate with no specific interaction of peptides to form oligomers, which absorb sunlight efficiently and transfer the harvesting light energy rapidly to an acceptor at the chlorosomal surface. The supramolecular structures have not yet been identified, but their local interaction was determined from various investigations (2–4,7–11): a special intermolecular bonding of 13-C=O···H–O(3^1)···Mg(central) and π–π interaction of composite chlorophyllous π-systems.

We earlier reported the self-aggregation of synthetic zinc BChl-*c*/*d*/*e* derivatives including **Zn-4/6** (Fig. 1) in nonpolar organic solvents as chlorosomal models (12–16), indicating that linear location of hydroxy, keto-carbonyl groups and a central coordinative metal including Mg, Zn and Cd in a molecule is necessary for such a self-aggregation. Natural self-aggregative BChl-*c*/*d*/*e* have chlorin (= 17,18-dihydroporphyrin) π-systems, and we have found that a bacteriochlorin (= 7,8,17,18-tetrahydroporphyrin) moiety was also useful for its self-aggregation (17). In this study, we report on the synthesis of zinc 3^1-hydroxy-13^1-oxo-porphyrins **Zn-1/2** (Fig. 1) by modifying naturally occurring Chl-*a* and their self-aggregation. A few reports are available on self-aggregation of synthetic hydroxy- and carbonyl-functionated porphyrins prepared from artificial *meso*-arylporphyrins (18,19) and octaethylporphyrin (20), but this is the first example of chlorosomal self-aggregation of porphyrins based on the natural chlorophyllous pigments (21).

In this study, we used the following tentative nomenclature. Protochlorophylls refer to 17,18-dehydro-chlorophylls and are currently widely used; we therefore used the prefix ''proto'' for 17,18-dehydrogenated compounds. Protobacteriochlorophyll-*d* (= 17,18-dehydro-BChl-*d*) has a fully conjugated porphyrin π-system. Protobacteriopheophorbide-*d* possessing 8-ethyl and 12-methyl groups can be termed 3^1-hydroxy-phytoporphyrin on the basis of IUPAC-IUB nomenclature (22).

MATERIALS AND METHODS

Apparatus. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured with a Bruker AC-300 spectrometer (Rheinstetten, Germany); chemical shifts (δ) are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) as an internal reference. Fast atomic bombardment (FAB) mass spectra were measured with a JEOL GCmate II spectrometer (Akishima, Japan). Visible absorption and circular dichroism (CD) spectra were measured with a Hitachi U-3500 spectrophotometer (Tokyo, Japan) and a Jasco J-720W spectropolarimeter (Hachioji, Japan), respectively. Infrared spectra were measured at room temperature with a Shimadzu FTIR-8600 spectrophotometer (Kyoto, Japan); a solution was measured in a KBr cell and a solid film on a thin aluminum-coated glass plate by means of reflection absorption spectroscopy through a Shimadzu AIM-800R microscope. High-performance liquid chromatography (HPLC) was done with a Shimadzu LC-10AS pump, an SPD-10AV visible detector and a C-R6A chromatopac. Flash column chromatography (FCC) was performed with silica gel (Merck, Kieselgel 60, 9385 Darmstadt, Germany).

Chemicals. Tetrahydrofuran (THF) was distilled from CaH_2 before use. Solvents for visible and CD spectra were purchased from Nacalai Tesque (spectroscopy grade, Kyoto, Japan). Methyl pyropheophorbide-*a* (**3**) (12,13), zinc methyl pyropheophorbide-*a* (**Zn-3**) (23), methyl bacterio-

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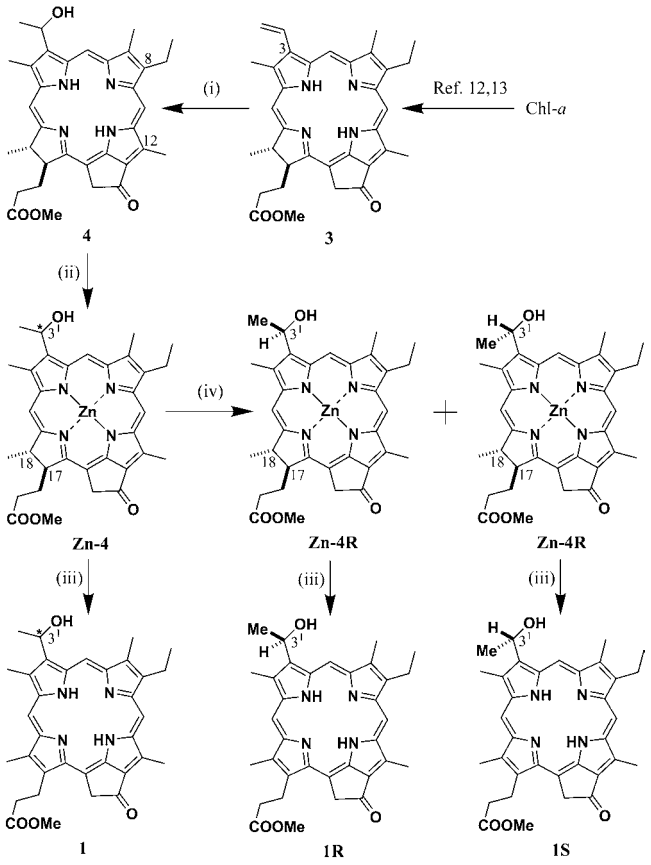
Abbreviations: BChl, bacteriochlorophyll; CD, circular dichroism; Chl, chlorophyll; DDQ, 2,3-dichloro-5,6-dicyano-benzoquinone; FAB, fast atomic bombardment; FCC, flash column chromatography; FWHM, full width at half-maximum; HPLC, high-performance liquid chromatography; ^{1}H NMR, proton nuclear magnetic resonance; THF, tetrahydrofuran.



Scheme 1. Synthesis of methyl protobacteriopheophorbide-*d* (**1**) and its 3^1-epimers **1R**/**1S**; (i) HBr–AcOH, H_2O, CH_2N_2–Et_2O; (ii) $Zn(OAc)_2 \cdot 2H_2O$/MeOH–CH_2Cl_2; (iii) DDQ–CH_3COCH_3, aq. HCl–CH_2Cl_2; (iv) reverse-phase HPLC separation.

pheophorbide-*d* (**4**) (13), zinc methyl bacteriopheophorbide-*d* (**Zn-4**) (13), zinc methyl 3^1R-bacteriopheophorbide-*d* (**Zn-4R**) (13), zinc methyl 3^1S-bacteriopheophorbide-*d* (**Zn-4S**) (13), methyl pyropheophorbide-*d* (**5**) (12), methyl 3^1-demethyl-bacteriopheophorbide-*d* (**6**) (12), zinc methyl 3^1-demethyl-bacteriopheophorbide-*d* (**Zn-6**) (12), methyl protopyropheophorbide-*a* (**7**) (23) were prepared according to the reported procedures. Other cyclic tetrapyrroles were synthesized in the following.

Methyl protobacteriopheophorbide-d *(**1**).* According to reported procedures (23), **Zn-4** was transformed to **1** as follows. To a distilled acetone solution (40 mL) of **Zn-4** ($3^1R/S$ = 1/1, 67 μmol, 42.1 mg) was slowly added a distilled acetone solution (20 mL) of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ; 100 μmol, 27.7 mg), and the reaction mixture was stirred at room temperature. After the disappearance of the 650 nm peak characteristic of the chlorin moiety, the reaction mixture was quenched with aq. 4% $KHSO_4$ and extracted with dichloromethane. The combined organic phases were washed with aq. 4% $NaHCO_3$ and water. After treatment of the organic layer with concentrated HCl for about 1 min with stirring at room temperature, the reaction mixture was quenched with aq. 4% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated *in vacuo*. The resulting residue was purified with FCC (1.5% MeOH–CH_2Cl_2) to give pure **1** ($3^1R/S$ = 1/1, 45 μmol, 25.2 mg) in 67% yield; see spectral data of **1** in Ref. 23.

Alternatively, the 3-vinyl group of **7** was hydrated by treatment of 30% HBr in acetic acid at 60°C for 3 h (to 3-CHBrMe), water [to 3-CH(OH)Me] and ethereal diazomethane (the resulting 17^2-COOH to 17^2-COOMe) (see (i) in Scheme 1 and Refs. 13,24) to give a 3^1-racemic mixture (1:1) of **1** in 46% yield.

*Methyl 3^1*R*-protobacteriopheophorbide*-d *(**1R**).* Similar to synthesis of **1**, HPLC-separated **Zn-4R** was DDQ oxidized and treated with acid to give **1R**; VIS (THF) λ_{max} = 639 (relative intensity, 0.01), 585 (0.05), 561 (0.08), 521 (0.05), 429 (0.54), 418 (1.00) nm; MS (FAB) found: *m*/*z* 565. Calcd for $C_{54}H_{36}N_4O_4$: MH^+, 565.

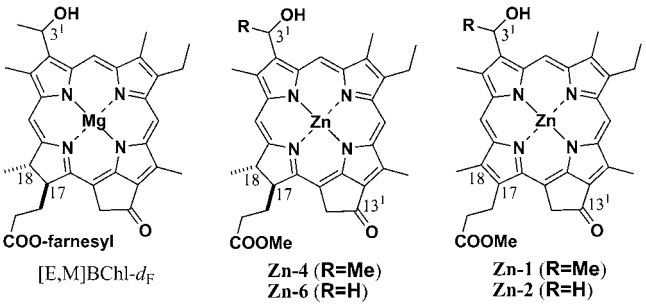


Figure 1. Molecular structures of a naturally occurring bacteriochlorophyll-*d* (left), its synthetic chlorin-type models **Zn-4/6** (center, 17CH–18CH) and the porphyrintype analogues **Zn-1/2** (right, 17C=18C).

*Methyl 3^1*S*-protobacteriopheophorbide*-d *(**1S**).* Similar to synthesis of **1**, HPLC-separated **Zn-4S** was DDQ oxidized and treated with acid to give **1S**; VIS (THF) λ_{max} = 639 (relative intensity, 0.01), 585 (0.05), 561 (0.08), 521 (0.05), 429 (0.54), 418 (1.00) nm; MS (FAB) found: *m*/*z* 565. Calcd for $C_{54}H_{36}N_4O_4$: MH^+, 565.

Methyl 3^1-demethyl-protobacteriopheophorbide-d *(**2**).* Zinc metallation of chlorin **5** (67 μmol, 36.9 mg) gave **Zn-5** (see below), which was DDQ oxidized and zinc demetallated (see above) to afford porphyrin **8** (23). The resulting residue containing **8** without purification by FCC was dissolved in dry dichloromethane (20 mL) and reduced by *t*-$BuNH_2BH_3$ (335 μmol, 29.1 mg). After stirring at room temperature for about 1 h, the reaction mixture was quenched with aq. 4% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated *in vacuo*. The resulting residue was purified with FCC (1% MeOH–CH_2Cl_2) to give pure **2** (35 μmol, 19.2 mg) in 52% total yield from **5**; **2**: melting point 258–260°C (from CH_2Cl_2 and hexane); VIS (THF) λ_{max} = 637 (relative intensity, 0.01), 584 (0.05), 562 (0.08), 521 (0.05), 430 (0.53), 417 (1.00) nm; see other spectral data of **2** in Ref. 23.

Zinc methyl protobacteriopheophorbide-d *(**Zn-1**).* To a dichloromethane solution of **1** ($3^1R/S$ = 1/1), a methanol solution saturated with zinc acetate dihydrate was added. After stirring at room temperature for 1 h, the reaction mixture was quenched with aq. 4% $NaHCO_3$. Produced white precipitates of zinc carbonate were filtered off. The filtrate was extracted with dichloromethane, and the combined organic layers were washed with water twice, dried over Na_2SO_4 and evaporated *in vacuo* to give pure **Zn-1** ($3^1R/S$ = 1/1) in an almost quantitative yield; dark green solid; melting point > 300°C (from CH_2Cl_2 and hexane); VIS (THF) λ_{max} = 607 (relative intensity, 0.11), 560 (0.05), 427 (1.00), 377 (0.12) nm; VIS (acetone) λ_{max} = 607 (ε, 2.5×10^4), 560 (1.7×10^4), 426 (2.5×10^5) nm; ^{1}H NMR ($CDCl_3$—3% pyridine-d_5) δ 10.15, 9.92, 9.61 (each 1H, s, 5-, 10-, 20-H), 6.57 (1H, q, *J* = 7 Hz, 3-CH), 5.65 (2H, s, 13^1-CH_2), 3.95 (2H, q, *J* = 8 Hz, 8-CH_2), 3.84, 3.72, 3.61, 3.47, 3.38 (each 3H, s, 2-, 7-, 12-, 18-CH_3, 17^2-$COOCH_3$), 3.92, 2.95 (each 2H, t, *J* = 8 Hz, 17-CH_2CH_2), 2.25 (3H, q, *J* = 7 Hz, 3-CH_3), 1.82 (3H, t, *J* = 8 Hz, 8^1-CH_3); MS (FAB) found: *m*/*z* 626. Calcd for $C_{54}H_{34}N_4O_4Zn$: M^+, 626.

*Zinc methyl 3^1*R*-protobacteriopheophorbide*-d *(**Zn-1R**).* Similar to the synthesis of **Zn-1**, **1R** was zinc metallated to give **Zn-1R**; VIS (THF) λ_{max} = 607 (relative intensity, 0.11), 560 (0.05), 427 (1.00), 377 (0.12) nm; MS (FAB) found: *m*/*z* 626. Calcd for $C_{54}H_{34}N_4O_4Zn$: M^+, 626.

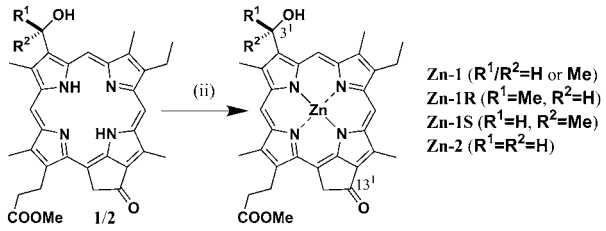
*Zinc methyl 3^1*S*-protobacteriopheophorbide*-d *(**Zn-1S**).* Similar to the synthesis of **Zn-1**, **1S** was zinc metallated to give **Zn-1S**; VIS (THF) λ_{max} = 607 (relative intensity, 0.11), 560 (0.05), 427 (1.00), 377 (0.12) nm; MS (FAB) found: *m*/*z* 626. Calcd for $C_{54}H_{34}N_4O_4Zn$: M^+, 626.

Zinc methyl 3^1-demethyl-protobacteriopheophorbide-d *(**Zn-2**).* Similar to the synthesis of **Zn-1**, **2** was zinc metallated to give **Zn-2**; VIS (THF) λ_{max} = 608 (relative intensity, 0.10), 590 (0.04, sh), 560 (0.05), 545 (0.04, sh), 524 (0.03), 428 (1.00), 406 (0.31, sh) nm; see other spectral data of **Zn-2** in Ref. 25.

RESULTS AND DISCUSSION

Synthesis of zinc methyl protobacteriopheophorbides-*d* Zn-1/2

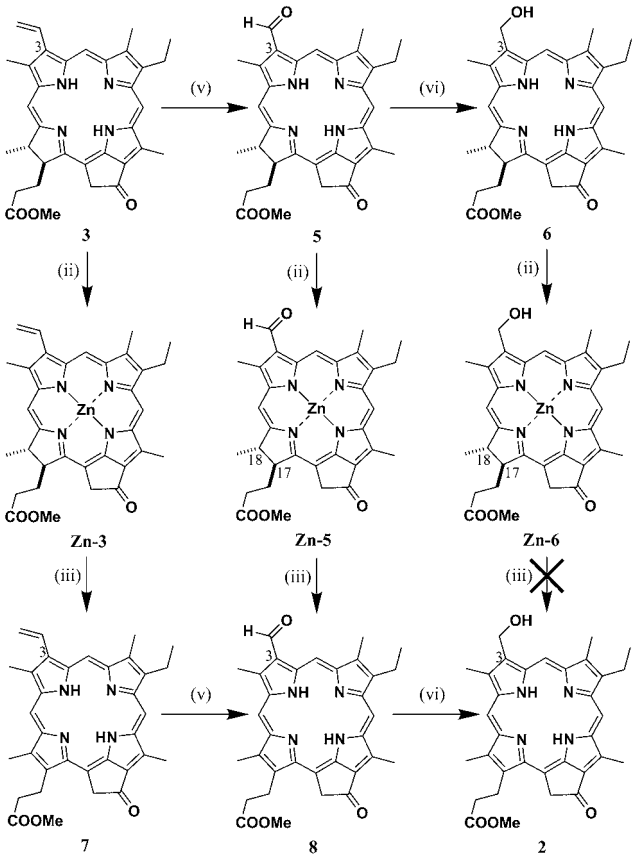
Methyl bacteriopheophorbide-*d* (**4**, see Scheme 1) possessing ethyl and methyl groups at the 8- and 12-positions, respectively, was



Scheme 2. Synthesis of zinc methyl protobacteriopheophorbides-*d*, **Zn-1** (3^1-epimeric 1:1 mixture), **Zn-1R** (3^1R-epimer) and **Zn-1R** (3^1R-epimer), and its 3^1-demethyl analog **Zn-2**; (ii) $Zn(OAc)_2 \cdot 2H_2O$/MeOH–CH_2Cl_2.

prepared by hydration of the 3-vinyl group in methyl pyropheophorbide-*a* (**3**), which was easily available by modifying Chl-*a* (13). After standard zinc metallation, zinc complex of **4**, **Zn-4,** was given as a 3^1-epimeric mixture (1:1) (13). According to the reported oxidation by DDQ in acetone (23), **Zn-4** (chlorin = *trans*-17,18-dihydroporphyrin) was transformed to the corresponding porphyrin and successive demetallation gave methyl protobacteriopheophorbide-*d* (**1**) as a pure form after purification by FCC. It is noted that direct oxidation of **4** to **1** could not occur by the action of DDQ because **4** was less electrochemically oxidized than **Zn-4** (23). The resulting **1** was a 1:1 racemic mixture at the 3^1-position. The racemate **1** was analytically separated by chiral HPLC (26), but the preparative separation of **1** to **1R** and **1S** was very difficult in this stage. On the other hand, a 3^1-epimeric mixture of **Zn-4** was separated by a single run of reverse-phase HPLC to afford **Zn-4R** and **Zn-4S** in a mg order (13). Each separated epimer **Zn-4R/S** was 17,18-dehydrogenated by DDQ and successively demetallated by an acid to give the corresponding metal-free porphyrin **1R/1S**, whose stereochemistry was determined by chiral HPLC. During the procedures, no change in the 3^1-stereochemistry was observed, and thus, racemically pure **1R** and **1S** were obtained in a preparative scale. Finally, zinc metallation of **1**(**R/S**) gave desired zinc methyl protobacteriopheophorbide-*d* [**Zn-1**(**R/S**), see Scheme 2] with retention of the 3^1-stereochemistry. Such zinc porphyrins were once produced after 17,18-dehydrogenation of the zinc chlorins described above, but little could be purified because of their lower solubility in usual organic solvents. Therefore, after demetallation of the oxidized products, pure free base porphyrins were isolated by purification of FCC. Their remetallation gave pure **Zn-1**(**R/S**) by recrystallization alone because zinc metallation proceeded smoothly and cleanly.

According to the similar access to (**Zn-**)**1** from 3-(1-hydroxyethyl)chlorin **4**, 3-hydroxymethyl-chlorin **6** (= 3^1-demethyl form of **4**, see Scheme 3), which was prepared by oxidation of the 3-vinyl group in **3** and successive reduction of the formyl group in the resulting methyl pyropheophorbide-*d* (**5**) (12), was applied to synthesis of the corresponding porphyrin **2**. After zinc metallation, DDQ oxidation of **Zn-6** gave a complex mixture as the product, and the desired corresponding porphyrin could not be detected in the mixture. The 3-hydroxymethyl group was so reactive that undesired overoxidation occurred at the less-sterically hindered benzylic position in **Zn-6** (primary alcohol) than in **Zn-4** (secondary alcohol). Therefore, **Zn-5** possessing a formyl group at the 3-position was similarly 17,18-dehydrogenated and demetallated to give 3-formyl-porphyrin **8**. The 3-formyl group was selectively reduced by *t*-$BuNH_2BH_3$ to give desired **2** (23) in a total yield of 43% from **3** to **2** via **Zn-5**. 3-Vinyl-chlorin **Zn-3** was also oxidized to give the porphyrin **7** after demetallation, and the transformation of the 3-vinyl group in **7** was transformed to the 3-hydroxymethyl



Scheme 3. Synthesis of methyl 3^1-demethyl-protobacteriopheophorbide-*d* (**2**); (ii) $Zn(OAc)_2 \cdot 2H_2O$/MeOH–CH_2Cl_2; (iii) DDQ–CH_3COCH_3, aq. HCl/CH_2Cl_2; (v) OsO_4/THF–$NaIO_4$/aq. AcOH; (vi) *t*-$BuNH_2BH_3$/CH_2Cl_2.

group in **2** through the 3-formyl group in **8** (23). The total yield from **3** to **2** via **Zn-3** was 39% (23), which was comparable to the former (43%), but the former was an easier route to **2** than the latter because all the present porphyrins were less soluble than the corresponding chlorins, and a large quantity of solvent was necessary for alternation of the peripheral substituents on the porphyrin π-system. It is noteworthy that **1** was alternatively afforded by hydration of the 3-vinyl group in porphyrin **7**, and the yield (46%) was less than that of hydration of chlorin **3** to **4** (82%).

Standard zinc metallation of **2** gave pure **Zn-2** quantitatively (Scheme 2). All 3^1-hydroxy-13^1-oxo-porphyrins (**Zn-**)**1**(**R/S**)/**2** were identified by their ^{1}H NMR, visible and/or mass spectra, indicating that they did not contain any chlorin moiety characterized by the intense Q_y-absorption band at around 650–660 nm in an organic solution.

Self-aggregation of zinc methyl protobacteriopheophorbide-*d* Zn-1(R/S)

A racemic mixture of **Zn-1** ($3^1R/S$ = 1/1) was dissolved in THF (0.2 m*M*) to give an intense absorption band at 427 nm as its Soret peak (broken line in Fig. 2). A relatively weak (one-ninth high) peak was observed at 607 nm, as its Q_y-absorption band possessing a full width at half-maximum (FWHM) of 350 cm^{-1}. These features indicated that **Zn-1** was monomeric in the THF solution. In 1%(vol/vol) THF and cyclohexane (0.2 m*M*), both the bands were shifted to longer wavelengths and broadened in comparison with the monomeric ones, as shown by the solid line in Fig. 2.

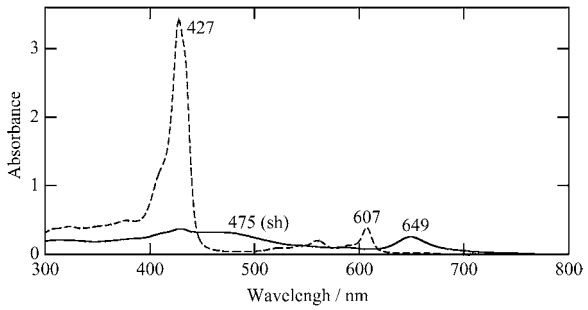


Figure 2. Visible spectra of 2×10^{-4} *M* **Zn-1** in THF (– – –) and 1%(vol/vol) THF and cyclohexane (—) in a 1 mm cell.

Typically, the Q_y band moved to 649 nm as its peak and had 810 cm^{-1} as its FWHM and a redshift of 1070 cm^{-1} and about a two-fold broadening occurred by decreasing polarity of the solvent (THF–cyclohexane = 100/0 → 1/99). The resulting Soret band was too broad to give an apparent peak but gave a shoulder at around 475 nm, whose absorbance intensity was as strong as that of the Q_y peak. The maximum at *ca* 430 nm was ascribed to residual monomer. Addition of THF to the self-aggregated solution gradually increased the monomeric bands and finally changed to the monomeric solution. Considering that similar visible changes were observed in self-aggregation of Chls (12,13), zinc porphyrin **Zn-1** self-aggregated in the nonpolar organic solvent.

In a THF solution of **Zn-1**, several vibrational bands were observed at 3410, 1742, 1699 and 1607 cm^{-1} (Fig. 3A), which were assigned to O–H, ester C=O, keto C=O and porphyrin skeletal C–C/C–N stretching, respectively, when compared with the reported data of chlorins (12,27–29). The values of these O–H and C=O vibrations showed that the functional groups were free from any specific interaction and also that **Zn-1** was monomeric in THF. In 1%(vol/vol) THF and cyclohexane, self-aggregates of **Zn-1** gave 1742, 1666 and 1607cm^{-1} vibrational peaks (Fig. 3B). A large shift in the keto-carbonyl stretching peak (1699 – 1666 = 33 cm^{-1}) by self-aggregation of porphyrin **Zn-1** is comparable with shifts by the formation of C=O···H–O···Zn in chlorin **Zn-4** (1691 – 1655 = 36 cm^{-1}). Therefore, **Zn-1** possessing a porphyrin π-system self-aggregated to form similar supramolecular structures with *in vivo* and *in vitro* self-aggregates of chlorosomal Chls (7–11) and their models of synthetic chlorins including **Zn-4** (13,15,27,30,31). Oligomeric **Zn-1** was built by special bondings, 13-C=O···H–O(3^1)···Zn, as well as π–π interaction of composite porphyrin moieties.

It is noteworthy that an 8 cm^{-1} higher wavenumber shift in monomeric 13-C=O stretching vibrational peaks was observed by 17,18-dehydrogenation as in **Zn-4** → **Zn-1**. This shift can be explained by the following. Change of the π-conjugation from chlorin to porphyrin enhances the steric repulsion between the 17-propionate and 13^2-H_2, and the 13-C=O in **Zn-1** was more out of the tetrapyrrole π-plane than that in **Zn-4**. Therefore, the 13-C=O in **Zn-1** was less conjugated with π-system of cyclic tetrapyrrole in a molecule and gave a higher wavenumber in the stretching vibration than that in **Zn-4**.

3^1-Racemically pure **Zn-1R/S** gave the same visible absorption bands as the 1:1 mixture **Zn-1** in THF and were monomeric species In 1%(vol/vol) THF and cyclohexane (0.2 m*M*), **Zn-1R** afforded the same visible spectrum as **Zn-1S**, as expected (Fig. 4A), but

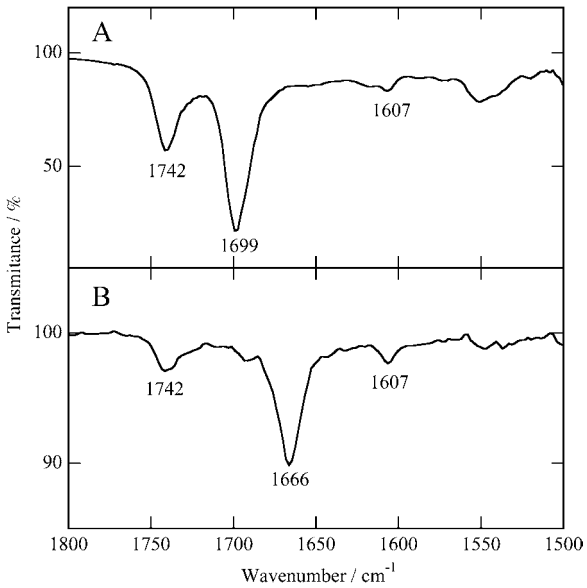


Figure 3. IR spectra of **Zn-1** in THF (A) and 1%(vol/vol) THF and cyclohexane (B) in a 0.1 and 0.5 mm KBr cell, respectively.

these spectra were slightly different from those of self-aggregates of **Zn-1** described above. The Q_y peaks of **Zn-1R/S** were situated at 652 nm and shifted to a 1140 cm^{-1} longer wavelength than the monomeric (607 nm). Their FWHMs were about 1000 cm^{-1} and larger than the value of **Zn-1** (810 cm^{-1}). The Soret peaks clearly appeared at 478 nm in **Zn-1R/S**. These visible absorption changes indicate that **Zn-1R/S** are similar self-aggregates in the nonpolar solvent with **Zn-1**. Slight differences show that **Zn-1** self-aggregated in the random order of the enantiomers, not in the separated manner similar to a 1:1 mixture of **Zn-1R** and **Zn-1S** self-aggregates.

In a THF solution (0.2 m*M*), neither of the enantiomers **Zn-1R/S** gave apparent CD peaks at 300–800 nm. This is reasonable because the 3^1-chiral functional group neither conjugates with the porphyrin

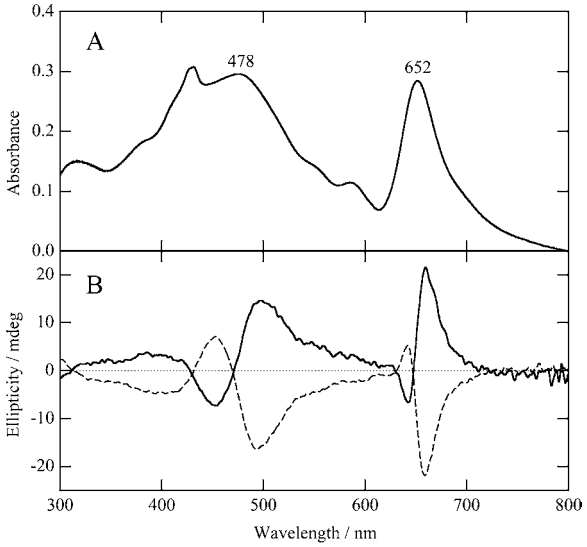


Figure 4. Visible spectrum (A) of 2×10^{-4} *M* **Zn-1R** and CD spectra (B) of 2×10^{-4} *M* **Zn-1R** (—) and **Zn-1S** (– – –) in 1%(vol/vol) THF and cyclohexane in a 1 mm cell.

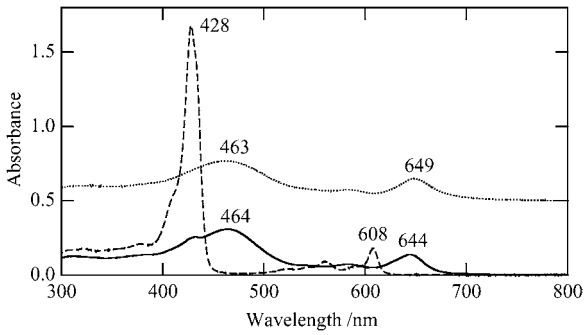


Figure 5. Visible spectra of 1×10^{-5} *M* **Zn-2** in THF (– – –) and 1%(vol/vol) THF and cyclohexane (—) in a 10 mm cell and of the solid film (· · ·) on a quartz glass.

π-system nor affects it from the steric aspect. In 1%(vol/vol) THF and cyclohexane (0.2 m*M*), **Zn-1R** afforded S-shaped CD peaks (+/–) at both the redshifted Q_y and Soret regions (solid line in Fig. 4B). This observation can be ascribed to the formation of ordered supramolecules by self-aggregation of chiral **Zn-1R**. **Zn-1R** self-aggregated in the nonpolar organic solvent to mainly form clockwise-twisted and helical supramolecular structures based on the exciton chirality method (32). In contrast, self-aggregates of **Zn-1S** gave reverse S-shapes (–/+) in the CD spectrum, which was a complete mirror image of that observed in **Zn-1R** self-aggregates (Fig. 4B). As expected, **Zn-1S** self-aggregated to form mirror-imaged (anticlockwise-twisted and helical) supramolecules of **Zn-1R** self-aggregates. (Very recently, Balaban *et al*. have reported similar observation of CD spectra in self-aggregates of chiral porphyrins [33].) Generation of the CD activity by self-aggregation of the 3^1-chiral compounds is supported by the results that racemic mixture **Zn-1** gave no CD peaks in the self-aggregates and by the reports that both **Zn-4R/S** self-aggregates gave giant CD peaks (13,27).

Self-aggregation of zinc methyl 3^1-demethyl-protobacteriopheophorbide-*d* Zn-2

In THF (0.01 m*M*), **Zn-2** (3-hydroxymethyl) was a monomeric species possessing 428 and 608 nm peaks as Soret and Q_y maxima, respectively (broken line in Fig. 5), as well as **Zn-1** [3-(1-hydroxyethyl)]. The Soret band had 10 times greater intensity

Table 1. Visible absorption spectral data of zinc methyl (proto)bacteriopheophorbides-*d* **Zn-1(R/S)/2/4/6** in monomeric and oligomeric solutions: absorption maximum λ_{max} (nm), FWHM (cm^{-1}) and redshift Δ (cm^{-1}) of λ_{max} by self-aggregation [$= \{1/\lambda_{max}(\text{monomer}) - 1/\lambda_{max}(\text{aggregates})\} \times 10^7$]

	λ_{max} [FWHM]		
	THF	1%(vol/vol) THF–cyclohexane	Δ
Zn-1 ($3^1R/S$ = 1/1)	427	475 (sh)	≈2400
	607 [350]	649 [810]	1070
Zn-1R/Zn-1S	427	478	2500
	607 [350]	652 [≈1000]	1140
Zn-2	428 [970]	464	1810
	608 [320]	644 [920]	920
Zn-4 ($3^1R/S$ = 1/1)	645 [320]	705 [670]*	1300
Zn-6	647 [320]	741 [750]	1960

*In 1%(vol/vol) CH_2Cl_2–cyclohexane.

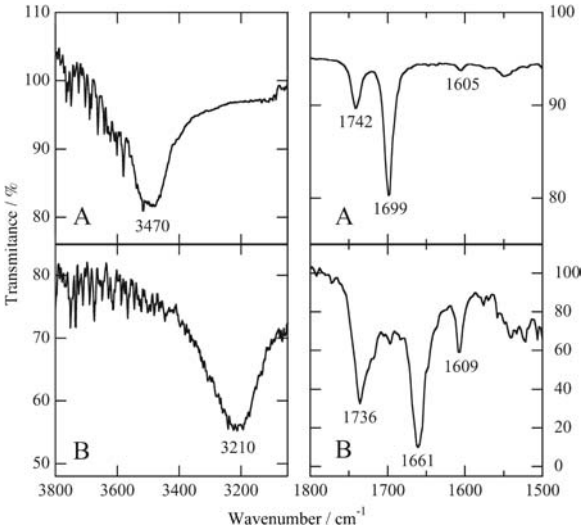


Figure 6. IR spectra of **Zn-2** in THF in a 0.1 mm KBr cell (A) and in the solid film on an aluminum-coated glass (B).

than the Q_y band. In 1%(vol/vol) THF and cyclohexane (0.01 m*M*), **Zn-2** self-aggregated to give similar oligomeric peaks at 464 and 644 nm with **Zn-1** self-aggregates (solid line in Fig. 5). The redshifted and broadened Soret band was twice as intense as the Q_y band. The self-aggregated Q_y band was redshifted in 920 cm^{-1} (608 → 644 nm) and about three times wider in the FWHM (320 → 920 cm^{-1}), compared with the monomeric ones. At 20 times lower concentration (0.01 m*M*), most of **Zn-2** self-aggregated to form large oligomers, whereas **Zn-1** remained monomeric. **Zn-2** (3-CH_2OH) was more self-aggregative than **Zn-1** [3-CH(OH)Me] because of the sterically less-hindered 3^1-hydroxy group, which is one of its most important interactive substituents (12,31). Therefore, **Zn-1** made oligomers more easily than **Zn-2** to form oligomeric precipitates just after preparation of the 0.2 m*M* solution. The resulting solid film gave exclusively oligomeric species possessing visible peaks at 463 and 649 nm and no residual monomeric peaks (dotted line in Fig. 5). It is noted that both self-aggregates of porphyrins **Zn-1/2** gave almost the same peak positions at around 650 nm (redshifts = *ca* 1000 cm^{-1}), whereas oligomers of more self-aggregative chlorins **Zn-6** (3-CH_2OH) (12) had a more redshifted Q_y band (647 → 741 nm, Δ = 1960 cm^{-1}) compared with oligomeric **Zn-4** [3-CH(OH)Me] (13) possessing a Q_y peak at 705 nm (← 645 nm, Δ = 1300 cm^{-1}) (see Table 1).

Monomeric **Zn-2** in THF gave 3470, 1742, 1699 and 1605 cm^{-1} vibrational bands (Fig. 6A), and oligomeric **Zn-2** in the solid film gave 3210, 1736, 1661 and 1609 cm^{-1} vibrational bands (Fig. 6B). Lower wavenumber shifts in 3^1-O–H and 13-C=O stretching vibrational bands (3470 – 3210 = 260 cm^{-1} and 1699 – 1661 = 38 cm^{-1}, respectively) by self-aggregation of **Zn-2** were comparable with values of 240/36 cm^{-1} in the corresponding chlorin **Zn-6** (12), indicating that oligomeric **Zn-2** had supramolecular structures similar to oligomeric **Zn-1/6**.

CONCLUSIONS

Zinc methyl protobacteriopheophorbides-*d* (**Zn-1/2**) possessing a porphyrin π-system were prepared by modifying naturally occurring Chl-*a* (via 17,18-dehydrogenation by DDQ) as models of

chlorosomal BChl-*d* possessing a chlorin π-system. Synthetic zinc porphyrins **Zn-1/2** self-aggregated in 1%(vol/vol) THF and cyclohexane to give large oligomers with a redshifted and broadened Q_y band at around 650 nm compared with the monomeric. The supramolecular structures of their oligomers were similar to those of the corresponding chlorins **Zn-4/6** as well as of chlorosomal self-aggregates, but the Q_y peaks were situated at a higher energy position than those of chlorin self-aggregates (see Table 1). Considering that zinc bacteriochlorins afforded similar self-aggregates (17) and 7-, 8- and 17-substitution did not disturb the formation of the self-aggregates (15,34–38), no alternation on the Q_x axis including the present formation of the 17,18-double bond affected self-aggregation of chlorosome-type pigments. Therefore, our previous finding (12,14) was supported: the 3^1-hydroxy, 13-ketocarbonyl groups and coordinative central metal on the Q_y axis are important for chlorosomal self-aggregation.

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REFERENCES

1. Senge, M. O. and S. Hatscher (2000) The malaria pigment haemozoin—a focal point of action for antimalarial drugs. *ChemBioChem.* **1**, 247–249.
2. Blankenship, R. E., J. M. Olson and M. Miller (1995) Antenna complexes from green photosynthetic bacteria. In *Anoxygenic Photosynthetic Bacteria* (Edited by R. E. Blankenship, M. T. Madigan and C. E. Bauer), pp. 399–435. Kluwer Academic Publishers, Dordrecht, The Netherlands.
3. Olson, J. M. (1998) Chlorophyll organization and function in green photosynthetic bacteria. *Photochem. Photobiol.* **67**, 61–75.
4. Blankenship, R. E. and K. Matsuura (2003) Antenna complexes from green photosynthetic bacteria. In *Light-Harvesting Antennas in Photosynthesis* (Edited by B. R. Green and W. W. Parson), pp. 195–217. Kluwer Academic Publishers, Dordrecht, The Netherlands.
5. Frigaard, N.-U., A. Gomez, M. Chew, H. Li, J. A. Maresca and D. A. Bryant (2003) *Chlorobium tepidum*: insight into the structure, physiology, and metabolism of a green sulfur bacterium derived from the complete genome sequence. *Photosynth. Res.* **78**, 93–117.
6. Saga, Y. and H. Tamiaki (2004) Fluorescence spectroscopy of single photosynthetic light-harvesting supramolecular systems. *Cell Biochem. Biophys.* **40**, 149–166.
7. Tamiaki, H. (1996) Supramolecular structure in extramembraneous antennae of green photosynthetic bacteria. *Coord. Chem. Rev.* **148**, 183–197.
8. Saga, Y., K. Matsuura and H. Tamiaki (2001) Spectroscopic studies on self-aggregation of bacteriochlorophyll-*e* in non-polar organic solvents: effects of stereoisomeric configuration at the 3^1-position and alkyl substituents at the 8^1-position. *Photochem. Photobiol.* **74**, 72–80.
9. van Rossum, B.-J., D. B. Steensgaard, F. M. Mulder, G. J. Boender, K. Schaffner, A. R. Holzwarth and H. J. M. de Groot (2001) A refined model of the chlorosomal antennae of the green bacterium *Chlorobium tepidum* form proton chemical shift constraints obtained with high-field 2-D and 3-D MAS NMR dipolar correlation spectroscopy. *Biochemistry* **40**, 1587–1595.
10. Mizoguchi, T., Y. Saga and H. Tamiaki (2002) Isolation and structure determination of a complete set of bacteriochlorophyll-*d* homologs and epimers from a green sulfur bacterium *Chlorobium vibrioforme* and their aggregation properties in hydrophobic solvents. *Photochem. Photobiol. Sci.* **1**, 780–787.
11. Umetsu, M., J. G. Hollander, J. Matysik, Z.-Y. Wang, T. Adschiri, T. Nozawa and H. J. M. de Groot (2004) Magic-angle spinning nuclear magnetic resonance under ultrahigh field reveals two forms of intermolecular interaction within CH_2Cl_2-treated (3^1R)-type bacteriochlorophyll *c* solid aggregate. *J. Phys. Chem. B* **108**, 2726–2734.
12. Tamiaki, H., M. Amakawa, Y. Shimono, R. Tanikaga, A. R. Holzwarth and K. Schaffner (1996) Synthetic zinc and magnesium chlorin aggregates as models for supramolecular antenna complexes in chlorosomes of green photosynthetic bacteria. *Photochem. Photobiol.* **63**, 92–99.
13. Tamiaki, H., S. Takeuchi, S. Tsudzuki, T. Miyatake and R. Tanikaga (1998) Self-aggregation of synthetic zinc chlorins with a chiral 1-hydroxyethyl group as a model for *in vivo* epimeric bacteriochlorophyll-*c* and *d* aggregates. *Tetrahedron* **54**, 6699–6718.
14. Yagai, S., T. Miyatake and H. Tamiaki (2002) Regio- and stereoisomeric control of the aggregation of zinc-chlorins possessing inverted interactive hydroxyl and carbonyl groups. *J. Org. Chem.* **67**, 49–58.
15. Tamiaki, H., M. Omoda, Y. Saga and H. Morishita (2003) Synthesis of homologously pure bacteriochlorophyll-*e* and *f* analogues from BChls-*c/d* via transformation of the 7-methyl to formyl group and self-aggregation of synthetic zinc methyl bacteriopheophorbides-*c/d/e/f* in non-polar organic solvent. *Tetrahedron* **59**, 4337–4350.
16. de Boer, I., J. Matysik, M. Amakawa, S. Yagai, H. Tamiaki, A. R. Holzwarth and H. J. M. de Groot (2003) MAS NMR structure of a microcrystalline Cd-bacteriochlorophyll *d* analog. *J. Am. Chem. Soc.* **125**, 13374–13375.
17. Kunieda, M., T. Mizoguchi and H. Tamiaki (2004) Diastereoselective self-aggregation of synthetic 3-(1-hydroxyethyl)-bacteriopyrochlorophyll-*a* as a novel photosynthetic antenna model absorbing near the infrared region. *Photochem. Photobiol.* **79**, 55–61.
18. Balaban, T. S., A. Eichhöfer and J.-M. Lehn (2000) Self-assembly by hydrogen bonding and π-π interactions in the crystal of a porphyrin—attempts to mimic bacteriochlorophyll c. *Eur. J. Org. Chem.* 4047–4057.
19. Balaban, T. S., A. D. Bhise, M. Fischer, M. Linke-Schaetzel, C. Roussel and N. Vanthuyne (2003) Controlling chirality and optical properties of artificial antenna systems with self-assembling porphyrins. *Angew. Chem. Int. Ed.* **42**, 2140–2144.
20. Tamiaki, H., S. Kimura and T. Kimura (2003) Self-aggregation of synthetic 2^1-hydroxy-12^1/13^1-oxo-porphyrins. *Tetrahedron* **59**, 7423–7435.
21. Tamiaki, H., T. Kubota and R. Tanikaga (1996) Aggregation of synthetic zinc complexes of cyclotetrapyrroles. *Chem. Lett.* 639–640.
22. Hynninen, P. H. (1991) Chemistry of chlorophylls: modifications. In *Chlorophylls* (Edited by H. Scheer), pp. 145–209. CRC Press, Boca Raton, FL.
23. Tamiaki, H., T. Watanabe and T. Miyatake (1999) Facile synthesis of 13^1-oxo-porphyrins possessing 3-vinyl or 3-formyl group, protochlorophyll-*a/d* derivatives by 17,18-dehydrogenation of chlorins. *J. Porphyrins Phthalocyanines* **3**, 45–52.
24. Smith, K. M., G. M. F. Bisset and M. Bushell (1980) Partial syntheses of optically pure methyl bacteriopheophorbides *c* and *d* from methyl pheophorbide *a*. *J. Org. Chem.* **45**, 2218–2224.
25. Tamiaki, H., T. Watanabe and M. Kunieda (2005) Self-aggregation of synthetic zinc 17,18-*cis*-bacteriochlorophyll-*d* derivative. In *Photosynthesis: Fundamental Aspects to Global Perspective* (Edited by A. der Est and D. Bruce) Allen Press, Lawrence, KS.
26. Tamiaki, H., M. Kouraba, K. Takeda, S. Kondo and R. Tanikaga (1998) Asymmetric synthesis of methyl bacteriopheophorbide-*d* and analogues by stereoselective reduction of the 3-acetyl to the 3-(1-hydroxyethyl) group. *Tetrahedron: Asymmetry* **9**, 2101–2111.
27. Balaban, T. S., H. Tamiaki, A. R. Holzwarth and K. Schaffner (1997) Self-assembly of methyl zinc (3^1R)- and (3^1S)-bacteriopheophorbides *d*. *J. Phys. Chem. B* **101**, 3424–3431.
28. Tamiaki, H., M. Amakawa, A. R. Holzwarth and K. Schaffner (2002) Aggregation of synthetic metallochlorins in hexane. A model of chlorosomal bacteriochlorophyll self-assemblies in green bacteria. *Photosynth. Res.* **71**, 59–67.
29. Yagai, S. and H. Tamiaki (2001) Synthesis and self-aggregation of zinc chlorophylls possessing an ω-hydroxyalkyl group: effect of distance between interactive hydroxy group and chlorin moiety on aggregation. *J. Chem. Soc. Perkin Trans.* **1**, 3135–3144.
30. Tamiaki, H., H. Kitamoto, A. Nishikawa, T. Hibino and R. Shibata (2004) Determination of 3^1-stereochemistry in synthetic bacteriochlorophyll-*d* homologs and self-aggregation of their zinc complexes. *Bioorg. Med. Chem.* **12**, 1657–1666.

31. Yagai, S., T. Miyatake, Y. Shimono and H. Tamiaki (2001) Supramolecular structure of self-assembled synthetic zinc-13^1-oxo-chlorins possessing a primary, secondary or tertiary alcoholic 3^1-hydroxyl group: visible spectroscopic and molecular modeling studies. *Photochem. Photobiol.* **73**, 153–163.
32. Pescitelli, G., S. Gabriel, Y. Wang, J. Fleischhauer, R. W. Woody and N. Berova (2003) Theoretical analysis of the porphyrin—porphyrin exciton interaction in circular dichroism spectra of dimeric tetraarylporphyrins. *J. Am. Chem. Soc.* **125**, 7613–7628.
33. Balaban, T. S., M. Linke-Schaetzel, A. D. Bhise, N. Vanthuyne and C. Roussel (2004) Green self-assembling porphyrins and chlorins as mimics of the natural bacteriochlorophylls *c, d*, and *e*. *Eur. J. Org. Chem.* 3919–3930.
34. Tamiaki, H., S. Miyata, Y. Kureishi and R. Tanikaga (1996) Aggregation of synthetic zinc chlorins with several esterified alkyl chains as models of bacteriochlorophyll-*c* homologs. *Tetrahedron* **52**, 12421–12432.
35. Tamiaki, H., M. Kubo and T. Oba (2000) Synthesis and self-assembly of zinc methyl bacteriopheophorbide-*f* and its homolog. *Tetrahedron* **56**, 6245–6257.
36. Oba, T. and H. Tamiaki (2001) Effect of C7-substituent on self-assembly of chlorosomal chlorophylls. *Supramol. Chem.* **12**, 369–378.
37. Sasaki, S., M. Omoda and H. Tamiaki (2004) Effects of C8-substituents on spectroscopic and self-aggregation properties of synthetic bacteriochlorophyll-*d* analogues. *J. Photochem. Photobiol. A: Chem.* **162**, 307–315.
38. Sasaki, S. and H. Tamiaki (2004) Self-assembly of synthetic bacteriochlorophyll-*f* analogues possessing C8-formyl group. *Bull. Chem. Soc. Jpn.* **77**, 797–800.