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Self-Aggregation of Synthetic Zinc Chlorins with a Chiral 1-Hydroxyethyl Group as a Model for *in vivo* Epimeric Bacteriochlorophyll-*c* and *d* Aggregates

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Abstract: 3^1-Epimerically pure zinc 3-(1-hydroxyethyl)-13^1-oxochlorins possessing several substituents at the 20-position were prepared. In non-polar organic solvents, the synthetic zinc complexes self-aggregated to form oligomers with >700-nm absorption and giant CD peaks, which were dependent upon the 3^1-absolute configuration as well as the 20-substituents. The *in vitro* self-aggregates of each epimeric zinc chlorin with a 20-methyl group showed similar visible and CD spectra with the *in vivo* bacteriochlorophyll-*c* ($3^1R/S$=2/1) aggregates in extramembranous antennae of a green photosynthetic bacterium. The spontaneous *in vitro* self-aggregates of $3^1R/S$(=2/1)-epimeric mixture of the zinc 20-methylchlorins were different from the natural supramolecules, indicating that *in vivo* slow oligomerization of $3^1R/S$(=2/1)-bacteriochlorophylls-*c* induced the regular supramolecular structures and/or the epimerically separated assemblies.

INTRODUCTION

Green photosynthetic bacteria have unique light-harvesting antenna systems (=chlorosomes). In natural chlorosomes, major chlorophyllous pigments self-aggregate to form oligomers which are characteristic of red-shifted absorption bands compared with the corresponding monomers.[1] The pigments are several homologues of magnesium complexes of 3^1-hydroxy-13^1-oxochlorins (=bacteriochlorophyll(=BChl)s-*c* and *d*, see Chart 1). BChls-*c* and *d* represent 20-methyl (R^{20}=Me) and 20-unsubstituted compounds (R^{20}=H), respectively. Natural BChls-*c*/*d* have a variety of substituents (=C_nH_m) at the 8- and 12-positions (R^8 and R^{12}) as well as the ester chain on the 17-propionate (R). The variation of the substituents affects the supramolecular structures of the self-aggregates and also energy migration in the oligomer. Several reports supported such controls in natural and model systems.[2] On the other hand, the absolute configuration at the chiral 3^1-position in the 3-(1-hydroxyethyl) group is dependent upon the bacteria species: typically BChl-*c* of a green filamentous gliding (non-sulfur) bacterium (Chloroflexaceae), *Chloroflexus aurantiacus* is a 2:1 mixture of 3^1R- and 3^1S-epimers.[3]

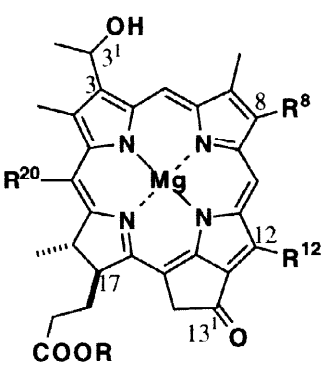

Chart 1. Chlorosomal chlorophylls
BChl-*c*: R^{20}=Me
BChl-*d*: R^{20}=H

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Recently, the diastereomeric control of the *in vitro* self-aggregates was reported: $3^1R/S$ epimerically pure samples gave different aggregates in organic solvents.[4] We know of few works[4e,f] which tried to elucidate the reasons why the 3^1-epimeric mixtures are in natural systems.

We reported earlier that synthetic zinc 3-hydroxymethyl-13^1-oxochlorins are good (stable and available) models for natural BChls-*c*/*d*.[5–7] Here we report on the synthesis of 3^1-epimerically pure zinc 3-(1-hydroxyethyl)-13^1-oxochlorins **3** and the self-aggregation of the 3^1-epimeric mixtures (several ratios, $3^1R/3^1S$ = 1/0 – 0/1) in non-polar organic solvents.

RESULTS AND DISCUSSION

Synthesis of Zinc Chlorins

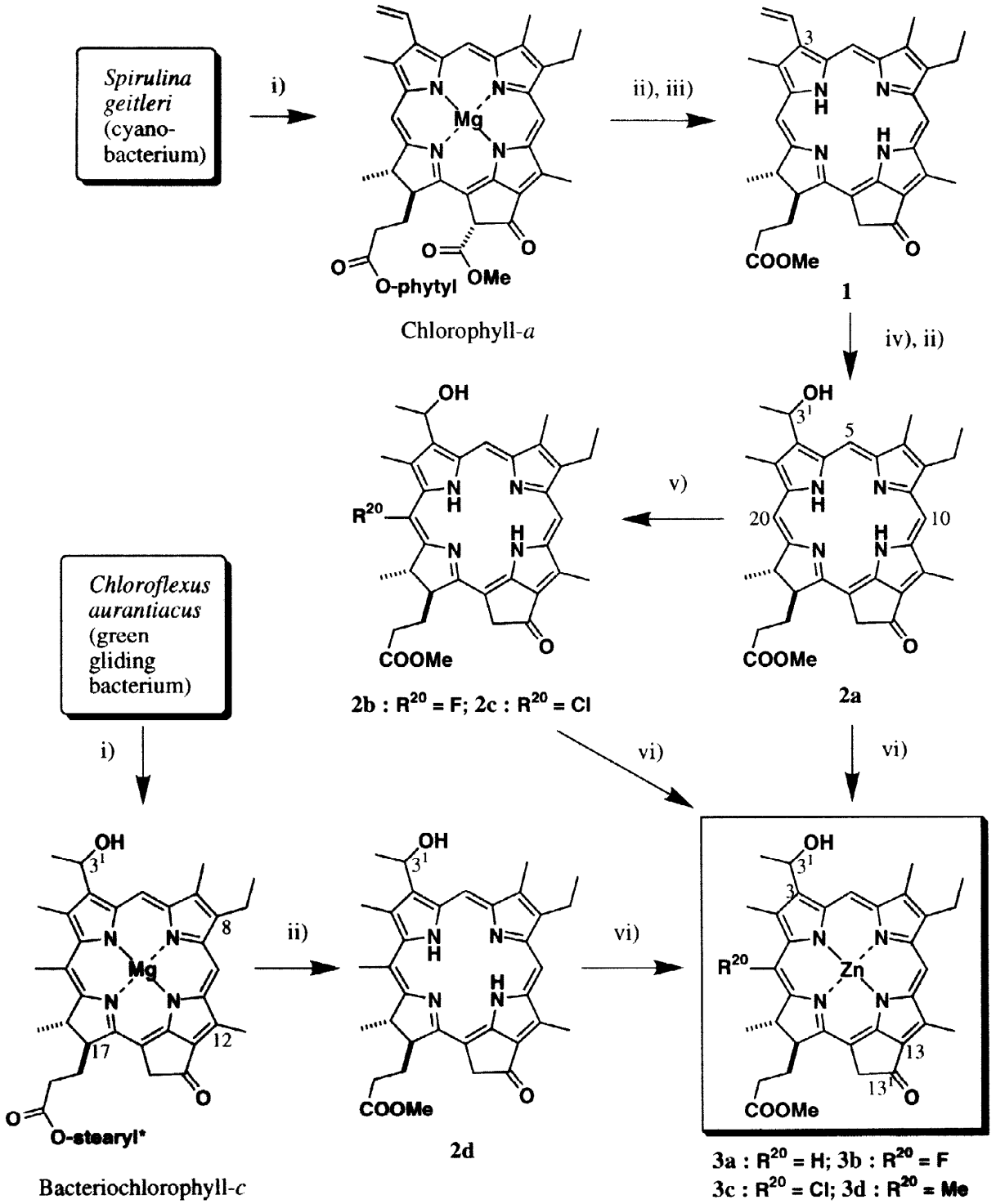
Chlorophyll(=Chl)-*a* was extracted from a cyanobacterium, *Spirulina geitleri* (see Scheme 1).[5] The commercially available species (used as carp feed, Dainippon Ink and Chemicals) has only Chl-*a* as a chlorophyllous pigment and Chl-*a* in the dry cell was more easily separated from other pigments than in higher plants (e.g., spinach) containing Chl-*b*. After treatment of the cell extract mixture by sulfuric acid in methanol, crude methyl pheophorbide-*a* was obtained by recrystallization from methanol and successive washing with water and hexane. The black solid was pyrolyzed and column chromatography and recrystallization gave pure methyl pyropheophorbide-*a* (**1**).[5] Hydration of the 3-vinyl group afforded methyl bacteriopheophorbide-*d* (**2a**) by slight modification of procedures originally reported by Smith and his colleagues (see Experimental section).[8] The reaction occurred non-diastereoselectively to lead an epimeric mixture of 3^1R (**2aR**) / 3^1S (**2aS**) = 1 / 1 (from ^{1}H-NMR spectral analysis). 20-Unsubstituted chlorin **2a** was halogenated according to our previous report[6] to give 20-fluoro / 20-chlorochlorins **2b** (R^{20}=F) and **2c** (R^{20}=Cl).

BChl-*c* was extracted from a green non-sulfur bacterium, *Chloroflexus aurantiacus* (see Scheme 1). The cultured species has only one homologue of BChl-*c* possessing ethyl and methyl groups at the 8- and 12-positions, respectively, except for a variation of 17-propionate ester chains and 3^1-chirality,[3] while green sulfur bacteria have many homologues possessing several alkyl groups at the 8- and 12-positions. The extracted BChl-*c* was changed to methyl ester of metal-free chlorin **2d**. The formula corresponds to 20-methylation of **2a**. Methyl bacteriopheophorbide-*c* (**2d**) prepared was an epimeric mixture of 3^1R (**2dR**) / 3^1S (**2dS**) = 2 / 1 because naturally occurring BChl-*c* in the chlorosome is an epimeric mixture of the same ratio.

The metal-free chlorins **2a-d** were metallated to give zinc complexes **3a-d**. Purification of flash column chromatography (FCC), recrystallization and HPLC (only MeOH as an eluant) gave 3^1-epimeric mixtures of pure zinc chlorins. All the synthetic chlorins were characterized by ^{1}H-NMR (1D and 2D-COSY/NOESY), visible and mass spectra.

Separation of Epimeric Chlorins

3^1-Epimeric mixtures of metal-free chlorins **2a** and **2d** have already been separated by several runs of reversed-phase HPLC (achieved upon recycling).[8] We also tried to separate the mixtures of all synthetic metal-free chlorins **2a-d** but were able to get only poorly separated chromatograms by a single run of HPLC.[9] However, an epimeric mixture (1:1) of zinc complex **3a** was easily separated by the single run (see Scheme 2). Under the same conditions (Cosmosil 5C_{18}-AR, 4.6 $\phi \times$ 250 mm, Nacalai Tesque, CH_3OH / H_2O = 4 / 1, 1.0



Scheme 1. Synthesis of zinc chlorins **3**

i) extraction; ii) H_2SO_4/MeOH; iii) collidine-reflux; iv) HBr/AcOH; v) $C_5H_5NF^+CF_3SO_4^-/CH_2Cl_2$–$C_6H_5CH_3$–$CH_3CN$ for fluorination, $NaClO_2$/H2O–THF for chlorination; vi) $Zn(OAc)_2$/MeOH–CH_2Cl_2.

* including other esters.[3]

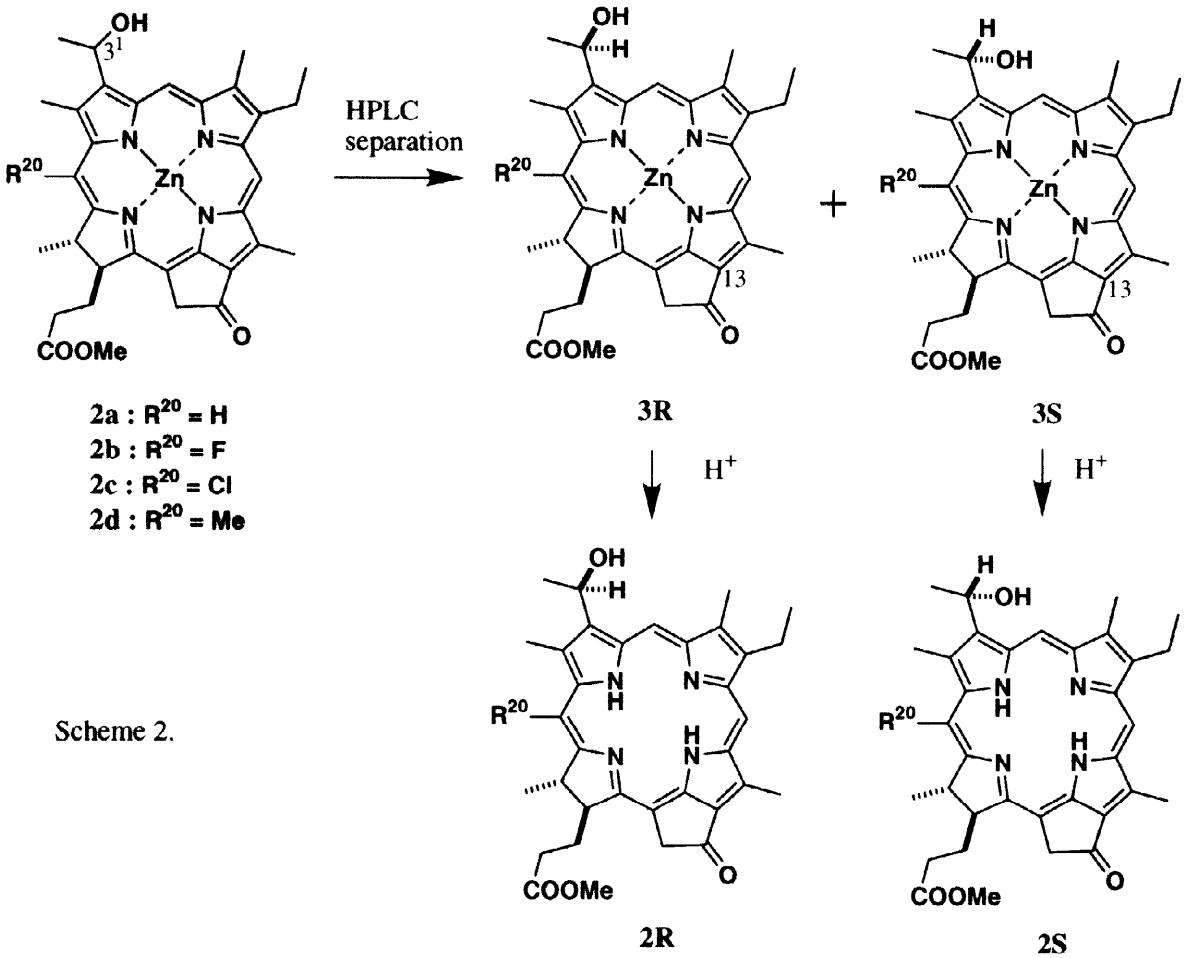
mL / min), 3^1-epimeric bands were completely separated (see Experimental section) and the separation ratio (R_S) between bands of (3^1R)-**3R** and (3^1S)-**3S** increased with 20-substitution: R_S = 1.9 (**3a**, R^{20}=H) < 2.6 (**3b**, R^{20}=F) < 2.9 (**3c**, R^{20}=Cl) < 3.0 (**3d**, R^{20}=Me). After preparative HPLC separation of the epimeric mixture of **3a**, each fraction was dried *in vacuo*. The separated zinc chlorins (**3aR / 3aS**) were demetallated by the action of acid to give 3^1-enantiomerically pure chlorins (**2aR / 2aS**) without epimerization at the 3^1-position (see Scheme 2).[10] Each metal-free chlorin in chloroform-*d* was analyzed by ^{1}H-NMR. Compared with reported chemical shifts at the *meso*-positions (5-, 10-, 20-H) of each stereochemistry-determined **2a**,[8] the metal-free chlorin derived from the first and second fractions was **2aR** and **2aS**, respectively, and then the first eluted band (retention time, rt = 32 min) was assigned to (3^1R)-**3aR** and the second band of rt = 34 min was (3^1S)-**3aS**.

Epimerically pure chlorins **2aR / 2aS** were fluorinated in an analytical scale (< 1 mg) and successively zinc-metallated as described above to afford epimeric zinc 20-fluorochlorins **3bR / 3bS** without epimerization, respectively. Each zinc chlorin was analyzed with HPLC, indicating that the first band (rt = 34 min) was **3bR** and the second band (rt = 39 min) was **3bS**. Moreover, in a preparative scale (> 10 mg), 20-unsubstituted chlorin **2aR** (or **2aS**) was fluorinated (*vide supra*) to give epimeric 20-fluorochlorin **2bR** (or **2bS**) stereoselectively and the first (or second) eluted band in HPLC of zinc fluorochlorin **3b** was demetallated to give epimerically pure **2b**. Comparison of the two ^{1}H-NMR spectra of the produced metal-free 20-fluorochlorins led to the same assignment as in HPLC analysis. Similarly, metal-free 20-unsubstituted chlorins **2aR / 2aS** were converted to zinc 20-chlorochlorins **3cR / 3cS** by chlorination and zinc-metallation. HPLC analysis showed that the first (rt = 31 min) and second bands (rt = 37 min) were **3cR** and **3cS**, respectively. Identification of ^{1}H-NMR spectra of **2cR** (or **2cS**) from chlorination of **2aR** (or **2aS**) with epimerically pure **2c** from demetallation of the first (or second) HPLC fraction of **3c** also supported the assignment derived from HPLC analysis.

Zinc methyl bacteriopheophorbide-*c* was a 2 : 1 mixture of **3dR / 3dS** as mentioned above. HPLC of the mixture gave the chromatogram that the 26-min band was 2-fold larger than the 29-min band. Therefore, the first eluted band was **3dR** and the second was **3dS**. In all four HPLC separations described here, the first fraction was (3^1R)-**3R** and the second was (3^1S)-**3S**. Moreover, each HPLC fraction separated from **3d** was demetallated to give epimerically pure **2d**. In comparing the observed ^{1}H-NMR signals of the produced **2d** with the corresponding reported data,[3] metal-free derivatives from the first and second bands were assigned to **2dR** and **2dS**, respectively.

Visible Spectra of Epimeric Zinc Chlorins

In a diluted dichloromethane solution (*ca.* 10 μM) of zinc chlorins **3** except **3bR** and **3cR**, all the visible absorption bands were sharp (see Fig. 1) and the widths of the Q_y band were typically about 400 cm^{-1}. Compared with previous results,[5] the visible spectra indicated that these compounds were monomeric in the solution. In **3bR** and **3cR**, a broad band appeared in the red side of the major monomeric Q_y band (see Fig. 2). The new band was ascribed to a small aggregate, probably a dimer. In zinc fluoro- and chlorochlorins, such a shoulder was clearly observed. The electron-withdrawing substituents at the 20-position would increase the coordination ability of the central zinc[11] so that a dimer species was formed even in a diluted dichloromethane solution. It is of interest that the (3^1R)-epimers **3bR** and **3cR** more easily aggregated to give such a dimer species than the corresponding (3^1S)-epimers (**3bS** and **3cS**). The diastereomeric control was



Scheme 2.

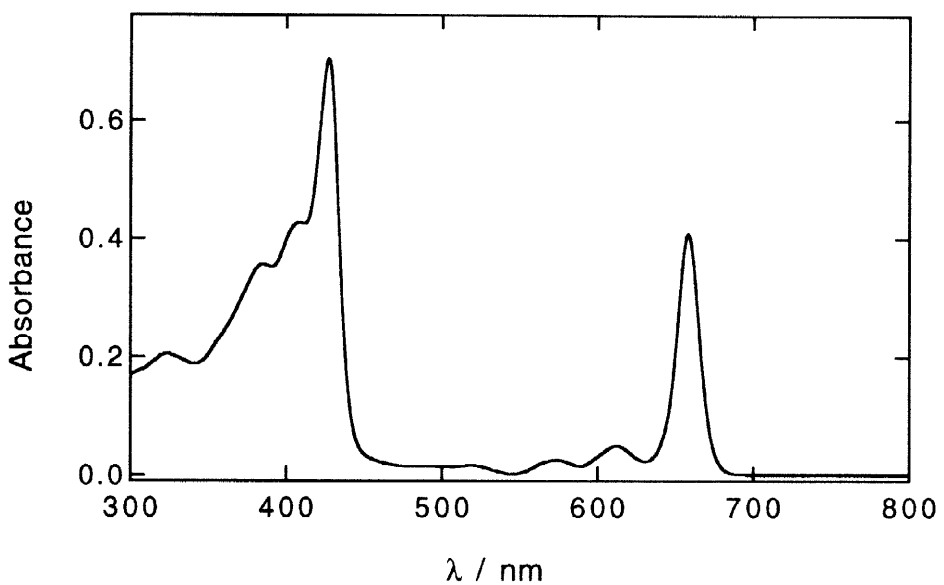


Fig. 1. UV-visible spectrum of zinc methyl 3¹*S*-bacteriopheophorbide-*c* (**3dS**) in dichloromethane (*ca.* 10 μM).

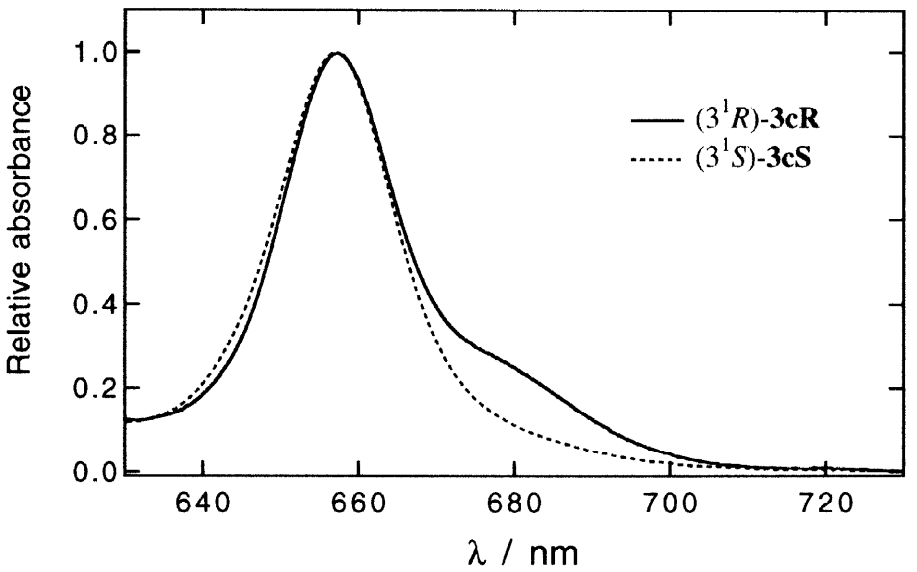


Fig. 2. Visible spectra of zinc methyl 20-chlorobacteriopheophorbide-*d* (**3cR / 3cS**) in dichloromethane (*ca.* 10 μM), normalized at Q_y peak.

consistent with our previous results in **3aR/S**.[4a] In tetrahydrofuran (THF), all the zinc chlorins **3** gave sharp visible bands and were solvated with THF to give monomeric species. The FT-IR spectra showed that a THF molecule coordinated with the central zinc and another hydrogen-bonded with the 3^1-hydroxyl group[5] (data not shown).

In monomeric species, both the epimers **3R/S** gave the same visible spectra and the Q_y peak was shifted in the order, **3a** (20-H) < **3b** (20-F) < **3c** (20-Cl) ≈ **3d** (20-Me) (see Table 1). The shift was ascribed to the steric effect at the 20-substituent and a similar shift was observed in the analogous system.[6] The molecular modeling (PM3/MM+ calculations[6]) indicated that the bulkiness of the 20-substituent distorted the chlorin π-plane, which induced red-shift of visible bands.

Table 1. Q_y maxima of epimeric zinc chlorins **3** (*ca.* 10 μM)

	R^{20}	λ_{max} / nm	
		CH_2Cl_2	1% (*v/v*) CH_2Cl_2 – cyclohexane[a]
3aR	H	648	703 [—, 705]
3aS	H	648	645, 698 [693, 706]
3bR	F	652, 672 (sh)	708 [—, 708]
3bS	F	652	647 (sh), 706 [—, 706]
3cR	Cl	658, 682 (sh)	736 [721, 743]
3cS	Cl	658	653 (sh), 716 [717, —]
3dR	Me	658	736 [—, 740]
3dS	Me	658	654, 720 [718, 732]

[a] Values in brackets show negative peaks in the second derivatives at the region of oligomeric Q_y bands.

In 1% (*v/v*) dichloromethane–cyclohexane, all the zinc complexes **3** gave broad and red-shifted absorption bands compared with the monomeric bands described above (see Fig. 3). The red-shifted Q_y peak was higher in intensity than the corresponding Soret peak, while the Q_y peak of monomeric species was lower than the monomeric Soret peak. The visible bands observed in non-polar organic solvents are characteristic of oligomeric **3** in comparison with other reported spectra.[5] The spectra of oligomeric (3^1R)-epimers **3R** were different from those of oligomeric (3^1S)-epimers **3S**; diastereomeric control was operated in the oligomerization of **3**. The Q_y peak of the oligomer in **3R** was more red-shifted than that in **3S**.[14] The second derivative of visible spectra indicated that oligomeric Q_y peaks consisted of several components. The major component of oligomeric **3R** absorbed longer wavelengths than that of oligomeric **3S**. In the visible spectra of **3R**, monomeric species could hardly be observed around 650 nm but **3S** gave a mixture of monomeric and oligomeric species. These results indicated that (3^1R)-epimers in the non-polar organic solvents self-aggregated more tightly to give longer wavelength absorbing oligomers than (3^1S)-epimers.

The oligomerization was achieved by dilution of a concentrated dichloromethane solution (*ca.* 1 mM) of **3** with excess cyclohexane. In such a high concentration of the dichloromethane solution, all the zinc chlorins **3** partly self-aggregated to give several small oligomers; typically, 1.4 mM dichloromethane solution of **3dR** or **3dS** had a new 699-nm absorption peak or 684-nm shoulder, respectively. The presence of these different aggregative species in the initial concentrated solution before dilution might lead to a different oligomerization in each 3^1-epimer, although this was ruled out by the following experiments. Addition of 0.5% (*v/v*) THF to the concentrated dichloromethane solution of **3** changed the visible spectra to completely monomeric sharp ones. Dilution of such a mixed solution including an exclusively monomer species with cyclohexane gave almost the same spectra as shown in Fig. 3. The results supported that oligomerization processes of each epimer **3R/S** in non-polar organic solvents were different as were the oligomerization products, which led to the different visible spectra in oligomeric **3R/S**. In all the oligomers of **3**, the local structure is the same and built-up mainly by 13-C=O···H(3-CHMe)O···Zn and π-π interaction of the chlorin chromophores reported so far.[4a,5,6] The size and/or the orientation of each component in the oligomers is dependent on the 3^1-configuration of the monomer and affects the supramolecular structures.

In the non-polar organic solution of unsubstituted and methylated (3^1S)-epimers **3aS** and **3dS**, monomeric peaks were clearly observed around the 650-nm region and **3bS** and **3cS** had less pronounced shoulders ascribable to the monomeric Q_y band. Zinc fluoro- and chlorochlorins aggregated more strongly than unsubstituted and methylated zinc chlorins. The electronic effect at the 20-substituent (*vide supra*) also operated in the oligomerization. Both the Q_y peaks of oligomer in **3R** and **3S** were shifted in the same order, **3a** < **3b** < **3c** ≈ **3d** as that of monomer. Steric bulkiness around the 20-position did not disturb the oligomerization.

Visible and CD Spectra of Epimeric Mixtures

A species of green non-sulfur bacteria, *Chloroflexus aurantiacus*, has several homologues of BChl-*c* as major antenna pigments in chlorosomes. The BChls-*c* consist of a mixture of (3^1R)- and (3^1S)-epimers (2:1); this prompted us to examine the properties in self-aggregates of 3^1-epimeric mixtures of the synthetic zinc chlorins **3**. A mixture of (3^1R)-**3dR** and (3^1S)-**3dS** (R^{20}=Me) at several ratios was dissolved in 0.5% (*v/v*) THF – dichloromethane to give a dark blue solution (*ca.* 1 mM) of only monomeric **3d** with a 660-nm absorption peak. The solution was diluted 100-fold with cyclohexane to make a green solution (*ca.* 10 μM).

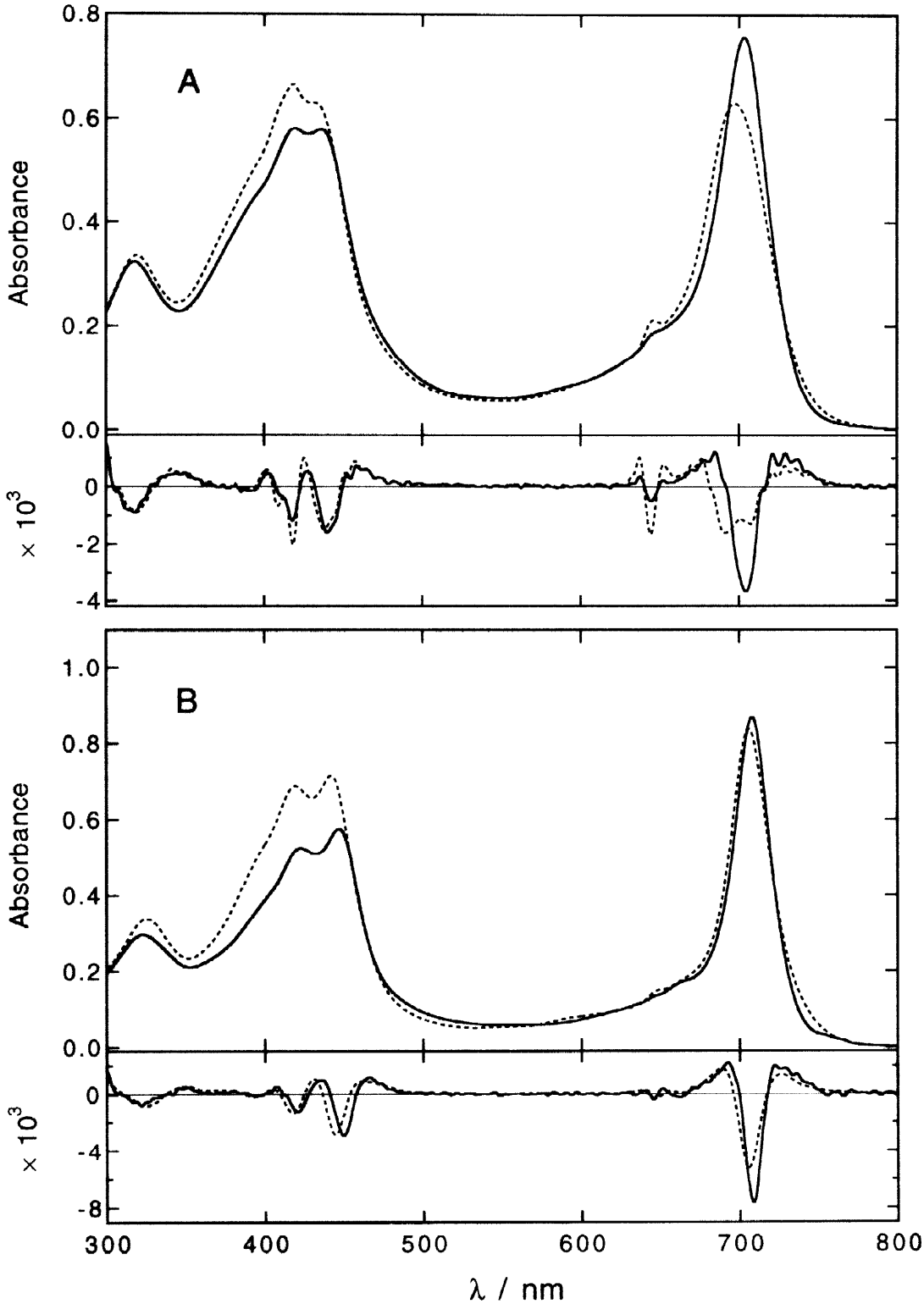


Fig. 3. UV-visible spectra and the second derivatives of epimerically pure **3** in 1% (*v*/*v*) CH_2Cl_2 – cyclohexane. ——, (3^1R)-epimer **3R**; ------, (3^1S)-epimer **3S**. A, **3a** (R^{20}=H); B, **3b** (R^{20}=F); C, **3c** (R^{20}=Cl); D, **3d** (R^{20}=Me).

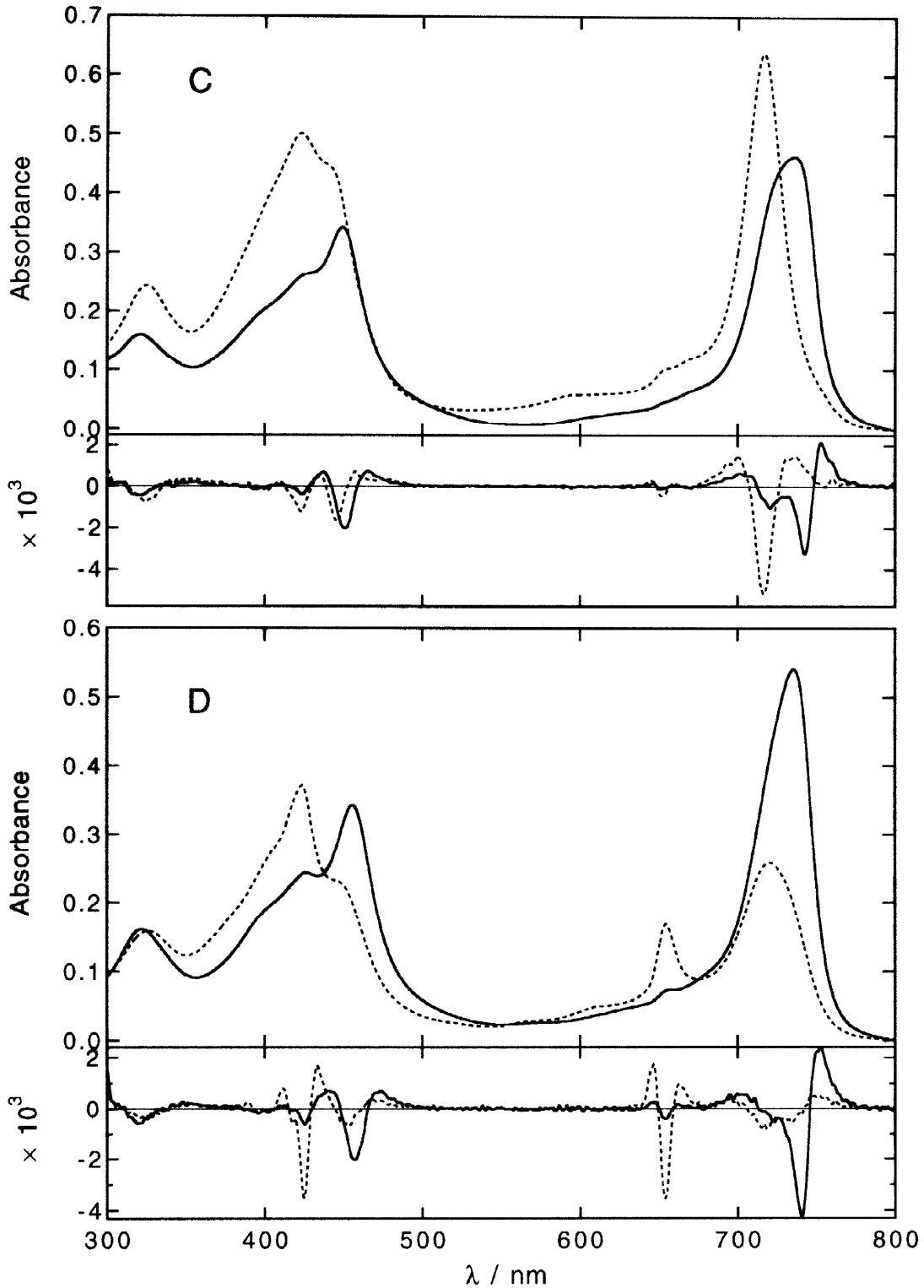


Fig. 3 (continued). UV-visible spectra and the second derivatives of epimerically pure **3** in 1% (*v*/*v*) CH_2Cl_2 – cyclohexane. ——, (3^1R)-epimer **3R**; ·····, (3^1S)-epimer **3S**. A, **3a** (R^{20}=H); B, **3b** (R^{20}=F); C, **3c** (R^{20}=Cl); D, **3d** (R^{20}=Me).

In 0.005% (*v*/*v*) THF–1% (*v*/*v*) dichloromethane–cyclohexane, **3d** self-aggregated to form oligomers absorbing above 700-nm wavelengths while the monomeric species (λ_{max}=654 nm) appeared as a minor portion (see Fig. 4). As the ratio of **3dS/3dR** increased, the monomer peaks enhanced concomitantly with decrease of the oligomer peaks (λ_{max}=715-740 nm). The (3^1*S*)-epimer **3dS** reduced the oligomerization of epimeric mixtures of **3d** as well as that of the epimerically pure sample discussed above. Moreover, the oligomer peaks were blue-shifted as the ratio of **3dS/3dR** increased. The second derivatives of the visible spectra in the region of Q_y band clearly indicated that a longer wavelength absorbing 740-nm component gradually changed to a shorter wavelength absorbing 718-nm component as the content of **3dS** increased in the epimeric mixture (see Fig. 5).

The CD spectra of **3dR** + **3dS** in the non-polar organic solvents are shown in Fig. 6. Both epimerically pure samples gave large CD peaks at the region of the red-shifted bands. In **3dR**, a reversed S-shaped band was observed with a minor dip at the shorter wavelengths, while the Cotton effect in **3dS** gave an exclusively similar reversed S-shape at a slightly blue-shifted region. Admixing an 3^1-epimer before dilution greatly decreased the intensity and slightly affected the shape. The order of the supramolecular structures was distorted as the ratio of a minor epimer increased (**3dR/3dS** = 1/0 → 1/1 or 0/1 → 1/1). (3^1*S*)-**3dS** as an admixing epimer affected the CD spectra more than (3^1*R*)-**3dR**. It is interesting that the oligomers of 2:1 – 1:2 mixtures of **3dR** and **3dS** gave smaller Cotton effects although they have similar visible spectra to oligomers of epimerically pure **3dS**. These results indicated that supramolecular structures of the oligomeric **3d** were dependent upon the 3^1-epimerical purity; **3dR**, **3dR** + **3dS** and **3dS** self-aggregated in the non-polar organic solvents to form slightly different supramolecules with the same local structure (13-C=O···H(3-CHMe)O···Zn and π-π interaction).

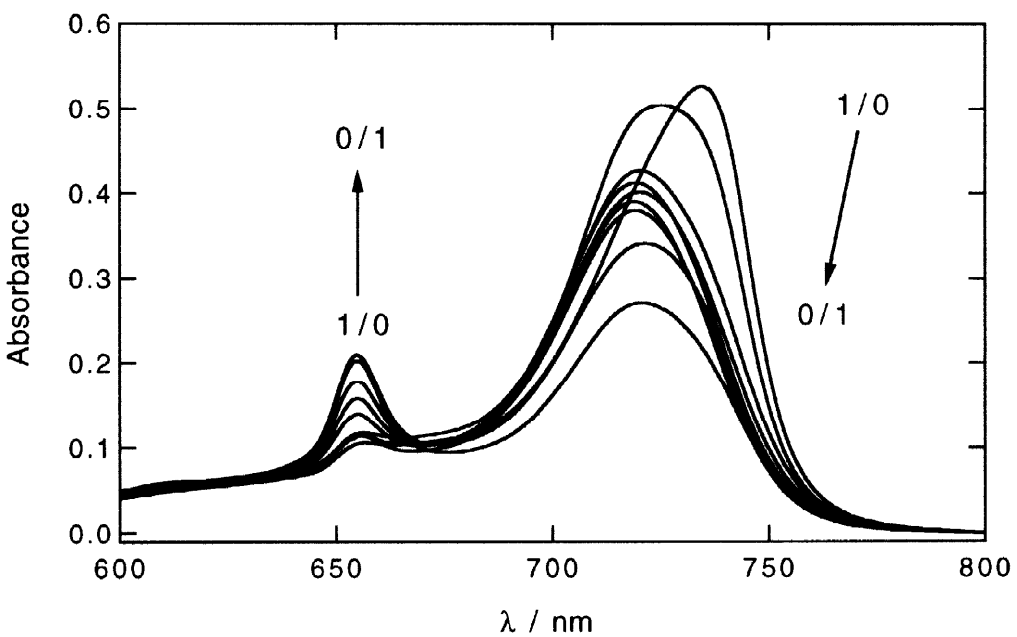


Fig. 4. Visible spectra of mixtures of (3^1*R*)-**3dR** and (3^1*S*)-**3dS** in 0.005% (*v*/*v*) – THF – 1% (*v*/*v*) CH_2Cl_2 – cyclohexane (*ca.* 10 μM).
3dR/3dS = 1/0, 12/1, 5/1, 2/1, 1/1, 1/2, 1/5, 1/12, 0/1.

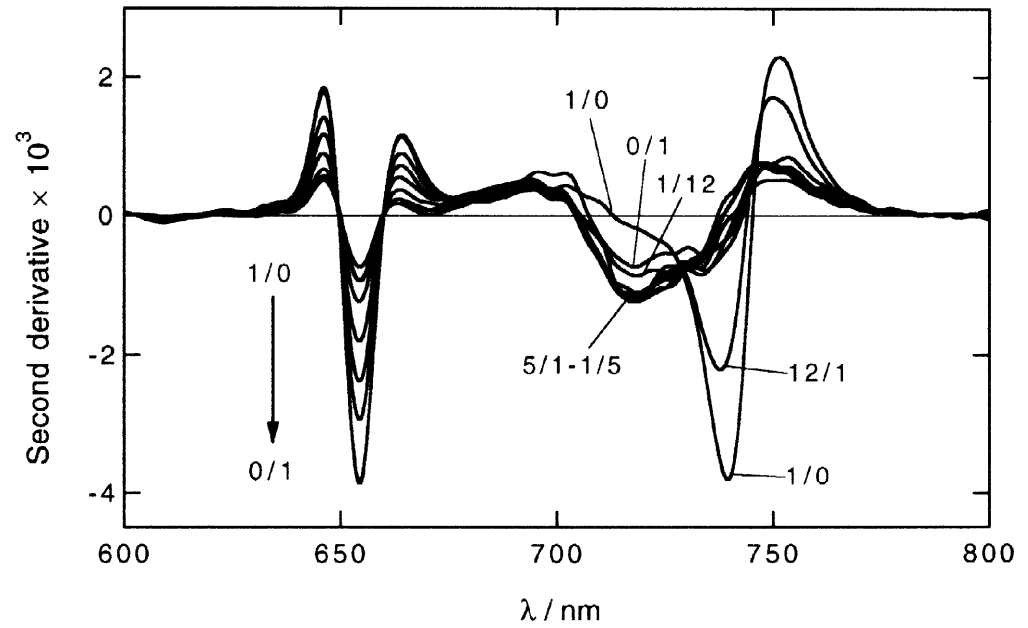


Fig. 5. Second derivatives of visible spectra of **3dR** + **3dS** mixtures in 0.005% (*v*/*v*) THF – 1% (*v*/*v*) CH_2Cl_2 – cyclohexane (*ca.* 10 μM).
3dR/3dS = 1/0, 12/1, 5/1, 2/1, 1/1, 1/2, 1/5, 1/12, 0/1.

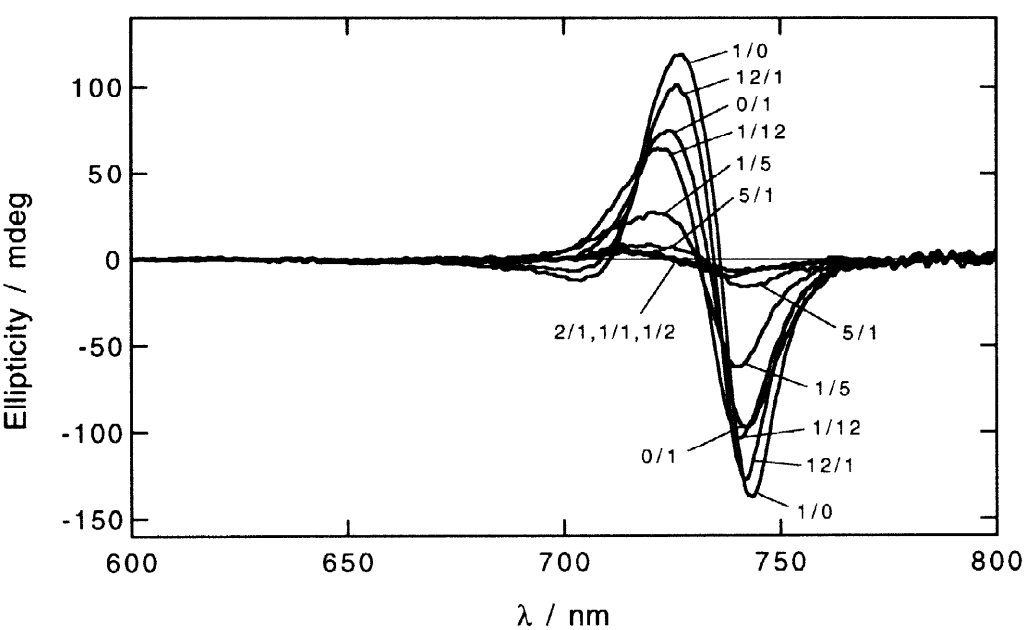


Fig. 6. CD spectra of **3dR** + **3dS** mixtures in 0.005% (*v*/*v*) THF – 1% (*v*/*v*) CH_2Cl_2 – cyclohexane (*ca.* 10 μM).
3dR/3dS = 1/0, 12/1, 5/1, 2/1, 1/1, 1/2, 1/5, 1/12, 0/1.

Comparison of *in vitro* Self-aggregates of Synthetic Zinc Chlorins with *in vivo* BChl-*c* Aggregate

Natural chlorosomes isolated from *Chloroflexus aurantiacus* in an aqueous buffer solution has a 741-nm peak as the red-shifted and broadened Q_y absorption and large reversed S-shape CD bands at the region with a minor negative peak at lower wavelengths (dotted lines of Fig. 7). The visible spectrum was more sharp and red-shifted than that of self-aggregates of **3dR/3dS** = 2/1 in the non-polar organic solvents (λ_{max}=720 nm in the broken line of Fig. 7A). The CD spectrum of chlorosomes was red-shifted to that of *in vitro* aggregates of the mixture as well. Moreover, the relative intensity was 4-fold higher than that of the spontaneous aggregates. These results indicated a difference in supramolecular structures between natural chlorosomes and the spontaneous self-aggregates of **3dR/3dS** = 2/1. The large Cotton effect in chlorosomes showed the orderly form of the self-aggregates of (3^1*R*)- and (3^1*S*)-BChls-*c* = 2/1 in chlorosomes. Rapid and efficient energy transfer in the chlorosomal aggregates[12] supported the regular self-assemblies. Self-aggregates of zinc (3^1*R*)- and (3^1*S*)-bacteriopheophytins-*c* (= 2/1) with long chains at the ester[13] gave small CD peaks in the non-polar organic solvents (data not shown). Substitution of the alkyl chain on the 17-propionate did not much affect the CD intensity in the spontaneous self-aggregates of the epimeric mixtures. Moreover, Miller and his colleagues[15] reported that aqueous aggregates of BChls-c (3^1*R*/*S* = 2/1) prepared by diluting a methanol solution of chloroform/methanol extracts from *Chloroflexus aurantiacus* with aqueous buffer showed 8-fold smaller Cotton effect than the intact chlorosomes. The reported suppression was consistent with the present results.

Initially self-aggregates of **3dR** and **3dS** were separately prepared in the non-polar organic solvents and then the solutions of the aggregates were mixed at a 2:1 ratio. The mixed solution after aggregation of the epimerically pure samples gave a sharper and red-shifted visible spectrum (λ_{max}=734 nm in the solid line of Fig. 7A) than the solution of aggregation of the epimeric mixture. The former solution showed 13-fold larger CD peaks than the latter (see the solid and broken lines of Fig. 7C). Both the visible and CD spectra indicated that the mixture of **3dR** aggregates/**3dS** aggregates = 2/1 was a better model as supramolecular structures of the chlorosomal aggregates of (3^1*R*)/(3^1*S*)-BChls-*c* = 2/1 than the aggregates of mixed **3dR/3dS** = 2/1. The visible spectrum of the mixture of the separated aggregates was superimposed to the sum of each spectrum (2:1) as well as the CD spectrum. The mixed solution after aggregation consisted of a mixture of separated aggregates, not random self-aggregates of the epimeric mixtures accumulated by the spontaneous aggregation of the mixtures. It can therefore be concluded that (3^1*R*)- and (3^1*S*)-BChls-*c* self-aggregated separately to form each oligomer in the chlorosomes. It is not necessary that the chlorosomes are completely separated into two parts, (3^1*R*)- and (3^1*S*)-BChls-*c* self-aggregates. It is enough that each epimerically pure oligomer unit occupying a small domain aggregates to form chlorosomal supramolecules. The presence of two components (or states) in chlorosomes was reported by analysis of CD,[16a,b] fluorescence anisotropy,[16b,c] linear dichroism[16d,e] and time-resolved emission/absorption spectra[16e-g] as well as modeling of the visible bands,[16h] which might support such epimerically separated aggregates as proposed here. In natural chlorosomes, BChls-*c* self-aggregate in the presence of other substances including lipids, quinones, carotenoids and peptides,[17] which might control the oligomerization. *In vivo* self-aggregation proceeds much more slowly than the spontaneous aggregation in the present system. Slow oligomerization of natural BChls-*c* (3^1*R*/*S* = 2/1) induced the regular supramolecular structures and/or the epimerically separated assemblies.

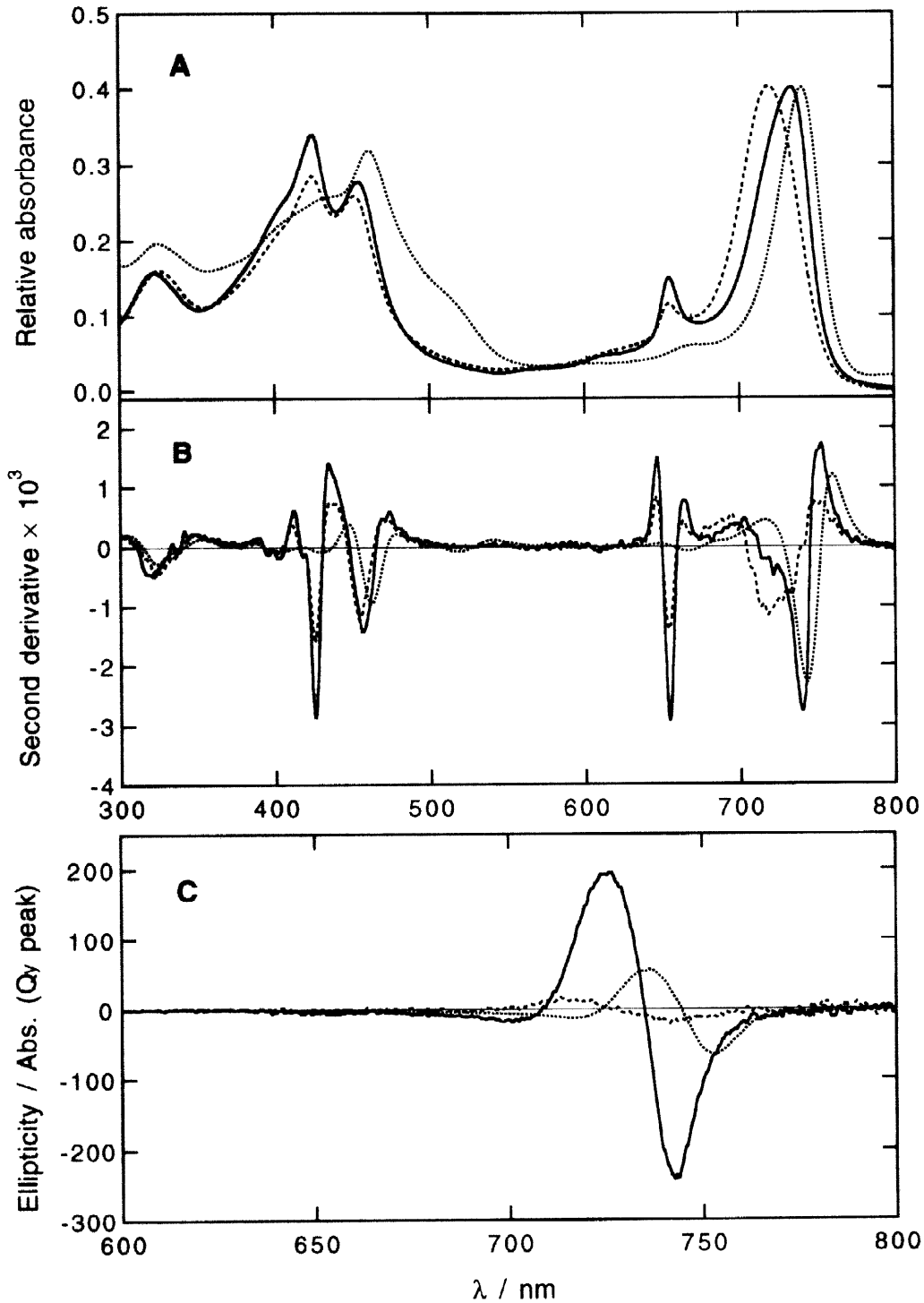


Fig. 7. UV-visible spectra normalized at Q_y peak (A), second derivatives of UV-visible spectra (B) and CD spectra normalized at Q_y absorption intensity (C).

....., isolated chlorosomes from *Chloroflexus aurantiacus* in 20 mM Tris-HCl buffer (pH=8);

-----, spontaneous self-aggregates of **3dR/3dS** = 2/1 in THF/CH_2Cl_2/cyclohexane = 0.005/1/99;

——, a mixture of **3dR**-aggregates and **3dS**-aggregates (=2/1) in THF/CH_2Cl_2/cyclohexane = 0.005/1/99.

EXPERIMENTAL

Apparatus

All of the apparatus used was the same as that described in our previous report.[18] Chemical shifts (δ) of ^{1}H-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.

Materials

Methyl pyropheophorbide-*a* (**1**) was prepared according to the procedures reported by Tamiaki and his colleagues.[5] Toluene and CH_3CN were distilled and stored over molecular sieves 3A. THF was 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, Hitachi Chemical Co., 6.0 ϕ × 150 mm or Cosmosil 5C_{18}-AR, 4.6 or 6.0 ϕ × 250 mm, Nacalai Tesque). Solvents for visible and CD spectra were purchased from Nacalai Tesque (Grade for UV-spectroscopy).

General

All procedures must be done in the dark!

Zinc Metallation of a Chlorin. To a saturated solution of $Zn(OAc)_2 \cdot 2H_2O$ in MeOH (2 mL), a solution of a metal-free chlorin **2** (0.03 mmol) in CH_2Cl_2 (10 mL) was added and stirred at room temperature under N_2 for 2 h. Aqueous 4% $NaHCO_3$ (15 mL) was added to the reaction mixture, stirred for 10 min, filtered, diluted with CH_2Cl_2, washed with H_2O and dried over Na_2SO_4. After evaporation of the solvents, the residue was purified by FCC (eluted with 0.5–1.5% MeOH / CH_2Cl_2), recrystallization from CH_2Cl_2 / hexane and HPLC (Gelpack GL-OP100) to give a pure zinc complex **3** (3^1-epimeric mixture). Each epimer **3R/3S** was separated by a single HPLC run (Cosmosil 5C_{18}-AR); a 4.6 mmϕ column for analytical separation (*vide supra*) and a 6.0 mmϕ column for preparative separation (*vide infra*).

Demetallation of a Zinc Chlorin. To a solution of a zinc chlorin **3** in THF, aqueous 5% HCl was added and stirred vigorously at room temperature under N_2 for 1.5 h. The reaction mixture was poured into ice-water, extracted with CH_2Cl_2. The organic phase was washed with aq. 4% $NaHCO_3$ and H_2O, dried over Na_2SO_4, evaporated and the residue was purified by FCC (2–5% ether / CH_2Cl_2) and recrystallization from CH_2Cl_2 / hexane to give the corresponding metal-free chlorin **2**.

Methyl Bacteriopheophorbide-*d* (2a)

The synthetic procedures reported by Smith and his colleagues[8] were slightly modified as follows. Methyl pyropheophorbide-*a* (**1**, 106 mg, 0.19 mmol) was dissolved in 30% hydrogen bromide in acetic acid (40 mL) and then stirred at 110 °C under N_2 for 1 h. The solution was poured into ice-water, extracted with $CHCl_3$, washed with H_2O three times, and dried over Na_2SO_4. After evaporation, the residue was dissolved in MeOH (50 mL) and to the solution was dropwise added conc. H_2SO_4 (5 mL) at 0 °C under N_2. After 1-h stirring at room temperature, the solution was poured into ice-water, extracted with $CHCl_3$ and washed with H_2O three times, and dried over Na_2SO_4. After evaporation, the residue was purified by FCC (0.2–0.4% MeOH / CH_2Cl_2) and recrystallization from CH_2Cl_2 / hexane to give 3^1-epimeric mixture of **2a** (94.2 mg, 86% yield, (3^1R) / (3^1S) = 1 / 1); black solids; mp 233-236 °C (lit,[8] 245-247 °C); VIS (CH_2Cl_2) λ_{max} = 659 (relative intensity, 0.43), 604 (0.07), 535 (0.09), 504 (0.09), 409 (1.00), 396 (0.79), 375 (0.56) nm; ^{1}H-NMR

($CDCl_3$) δ (*R*/*S*) = 9.67/69 (1H, s, 5-H), 9.49 (1H, s, 10-H), $8.51_5/50_7$ (1H, s, 20-H), 6.44/42 (1H, q, *J*=7 Hz, 3-CH), 5.25/24, $5.08_5/07_9$ (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.47 (1H, dq, *J*=2, 7 Hz, 18-H), 4.27 (1H, dt, *J*=8, 2 Hz, 17-H), 3.70 (2H, q, *J*=8 Hz, 8-CH_2), 3.65 (3H, s, 12-CH_3), $3.60_8/61_4$ (3H, s, 17^2-CO_2CH_3), 3.42/41 (3H, s, 2-CH_3), 3.25 (3H, s, 7-CH_3), 2.45-2.75, 2.20-2.35 (each 2H, m, 17-CH_2CH_2), 2.15 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.80/78 (3H, d, *J*=7 Hz, 18-CH_3), 1.69 (3H, t, *J*=7 Hz, 8^1-CH_3), 0.35 (1H, br, NH), -1.80 (1H, s, NH). MS (FAB) found: *m/z* 566. Calcd for $C_{34}H_{38}N_4O_4$: M^+, 566.

Methyl 20-Fluorobacteriopheophorbide-*d* (2b)

To a solution of methyl bacteriopheophorbide-*d* (**2a**, 45 mg, 0.079 mmol) in a minimum amount of CH_2Cl_2 (*ca.* 5 mL) and toluene (25 mL), a solution of *N*-fluoropyridinium triflate (190 mg, 0.77 mmol) in CH_3CN (5 mL) was added and stirred vigorously at 50 °C under N_2 for 1.5 h.[6] The reaction mixture was washed with aq. 2% HCl, aq. 4% $NaHCO_3$ and aq. saturated NaCl and dried over Na_2SO_4. After evaporation, the residue was purified by FCC (2–5% ether / CH_2Cl_2) and recrystallization from CH_2Cl_2 / hexane to give the fluorinated chlorin **2b** (5.1 mg, 11% yield, (3^1R) / (3^1S) = 1 / 1); black solids; mp 125-130 °C; VIS (CH_2Cl_2) λ_{max} = 664.5 (0.37), 606 (0.05), 542.5 (0.09), 510.5 (0.09), 409.5 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ (*R*/*S*) = 10.03/04 (1H, s, 5-H), 9.60 (1H, s, 10-H), 6.52/51 (1H, q, *J*=7 Hz, 3-CH), 5.32/31, 5.22/21 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.77/76 (1H, q, *J*=7 Hz, 18-H), 4.30-4.38 (1H, m, 17-H), 3.73 (2H, q, *J*=7 Hz, 8-CH_2), 3.72 (3H, s, 12-CH_3), 3.59/60 (3H, s, 17^2-CO_2CH_3), 3.55/56 (3H, d, J_{HF}=4 Hz, 2-CH_3), 3.32 (3H, s, 7-CH_3), 2.51-2.75, 2.10-2.36 (each 2H, m, 17-CH_2CH_2), 2.20/19 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.76/72 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.75/70 (3H, d, *J*=7 Hz, 18-CH_3), -2.88/89 (1H, s, NH^{19}). MS (FAB) found: *m/z* 585. Calcd for $C_{34}H_{38}N_4O_4F$: MH^+, 585.

Methyl 20-Chlorobacteriopheophorbide-*d* (2c)

To a solution of methyl bacteriopheophorbide-*d* (**2a**, 10 mg, 0.018 mmol) in THF (20 mL), a solution of $NaClO_2$ (1.5 mg, 3 mol) in H_2O (10 mL) was added, followed by a drop of conc. HCl and the mixture solution was stirred vigorously at room temperature under N_2 for 50 min.[6] The reaction mixture was poured into aq. 4% $NaHCO_3$, extracted with CH_2Cl_2, washed with H_2O twice and dried over Na_2SO_4. After evaporation, the residue was purified by FCC (2–3% ether / CH_2Cl_2) and recrystallization from CH_2Cl_2 / hexane to give the chlorinated chlorin **2c** (4.8 mg, 45% yield, (3^1R) / (3^1S) = 1 / 1); black solids; mp 130-135 °C; VIS (CH_2Cl_2) λ_{max} = 671.5 (0.48), 613.5 (0.06), 548.5 (0.13), 516 (0.08), 412.5 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ (*R*/*S*) = 10.02/07 (1H, s, 5-H), 9.42/39 (1H, s, 10-H), 6.48/44 (1H, q, *J*=7 Hz, 3-CH), 5.11, 5.02 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.77/74 (1H, q, *J*=7 Hz, 18-H), 4.05-4.23 (1H, m, 17-H), 3.69 (2H, q, *J*=7 Hz, 8-CH_2), 3.63 (3H, s, 12-CH_3), 3.60/59 (3H, s, 17^2-CO_2CH_3), 3.59/58 (3H, s, 2-CH_3), 3.28/27 (3H, s, 7-CH_3), 2.55-2.67, 2.20-2.45 (each 2H, m, 17-CH_2CH_2), 2.14/16 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.68 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.57/51 (3H, d, *J*=7 Hz, 18-CH_3), -2.12 (1H, s, NH^{19}). MS (FAB) found: *m/z* 600. Calcd for $C_{34}H_{37}N_4O_4{}^{35}Cl$: M^+, 600.

Methyl Bacteriopheophorbide-*c* (2d)

After complete cultivation of *Chloroflexus aurantiacus J-10-fl*,[20] the cultured solution (10 L) was centrifuged to give green bacteria by decantation. MeOH (200 mL) was added to the green bacteria (*ca.* 22 g) and the suspension was vigorously stirred for 1 h under Ar. After filtration, the residue was repeatedly extracted with MeOH / petroleum ether = 1 / 1 (200 mL), until the filtrate was colorless (three times at least).

1% (v/v) conc. HCl – brine (20 mL) was added to each filtrate and the organic phase was separated. The aqueous phase was re-extracted with several portions of diethyl ether / petroleum ether = 1 / 1 and the combined organic phases were washed with H_2O, and dried over Na_2SO_4. After evaporation, the residue was suspended in MeOH (100 mL), to which was added ice-chilled 20% (*v/v*) conc. H_2SO_4 – MeOH (100 mL) at 0 °C under N_2. After 1-h stirring, the solution was poured into ice-water, extracted with CH_2Cl_2, washed with H_2O several times, and dried over Na_2SO_4. After evaporation, the residue was reprecipitated from CH_2Cl_2 / hexane and washed with excess hexane to remove most orange carotenoids. The solids were purified by FCC to give carotenoids as an orange fraction (CH_2Cl_2), methyl bacteriopheophorbide-*a* as a pale red fraction (1% MeOH / CH_2Cl_2) and subsequently a major reddish black elution (2% MeOH / CH_2Cl_2). The main fraction was evaporated and recrystallized from CH_2Cl_2 / hexane to give the desired **2d**[3,8,21] (103 mg, (3^1*R*) / (3^1*S*) = 2 / 1); black solids; mp 94-96 °C; VIS (CH_2Cl_2) λ_{max} = 670 (0.44), 612 (0.09), 551 (0.15), 520 (0.12), 482 (0.05), 414 (1.00) nm; ^{1}H-NMR ($CDCl_3$) δ (*R/S*) = 9.90/92 (1H, s, 5-H), 9.45 (1H, s, 10-H), 6.50/48 (1H, q, *J*=7 Hz, 3-CH), 5.21/19 (2H, s, 13^1-CH), 4.57 (1H, q, *J*=7 Hz, 18-H), 4.16 (1H, dd, *J*=3, 8 Hz, 17-H), 3.87 (3H, s, 20-CH_3), 3.70 (2H, q, *J*=8 Hz, 8-CH_2), 3.65 (3H, s, 12-CH_3), 3.58/59 (3H, s, 17^2-CO_2CH_3), 3.49 (3H, s, 2-CH_3), 3.28 (3H, s, 7-CH_3), 2.45-2.60, 2.15-2.23 (each 2H, m, 17-CH_2CH_2), 2.13/15 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.69 (3H, t, *J*=8 Hz, 8^1-CH_3), 1.48/46 (3H, d, *J*=7 Hz, 18-CH_3), -1.89 (1H, s, NH[19]). MS (FAB) found: *m/z* 580. Calcd for $C_{35}H_{40}N_4O_4$: M^+, 580.

Spectral Data

Zinc Methyl Bacteriopheophorbide-*d* (3^1*R*/3^1*S*=1/1) (3a). Retention time was 5.3 min (Gelpack, MeOH, 1.5 mL / min); dark green solids; mp > 300 °C; VIS (CH_2Cl_2) λ_{max} = 648 (0.69), 603 (0.11), 555.5 (0.05), 512.5 (0.04), 422 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.49, 9.46, 8.36 (each 1H, s, 5-, 10-, 20-H), 6.27 (1H, q, *J*=7 Hz, 3-CH), 5.14, 5.10 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.44 (1H, q, *J*=7 Hz, 18-H), 4.17-4.25 (1H, m, 17-H), 3.72 (2H, q, *J*=7 Hz, 8-CH_2), 3.53, 3.34, 3.31, 3.22 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.55-2.70, 2.40-2.20 (each 2H, m, 17-CH_2CH_2), 2.08 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.78/77 (3H, d, *J*=7 Hz, 18-CH_3), 1.69 (3H, t, *J*=7 Hz, 8^1-CH_3). MS (FAB) found: *m/z* 628. Calcd for $C_{34}H_{36}N_4O_4{}^{64}Zn$: M^+, 628.

Zinc Methyl 3^1*R*-Bacteriopheophorbide-*d* (3aR). Retention time was 68 min (6 mmϕ Cosmosil, MeOH / H_2O = 3 / 1, 1.0 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 648 (0.66), 602.5 (0.11), 556.5 (0.06), 512.5 (0.05), 422 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.49, 9.43, 8.36 (each 1H, s, 5-, 10-, 20-H), 6.28 (1H, q, *J*=7 Hz, 3-CH), 5.12, 4.99 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.43 (1H, q, *J*=7 Hz, 18-H), 4.14-4.24 (1H, m, 17-H), 3.70 (2H, q, *J*=7 Hz, 8-CH_2), 3.52, 3.30, 3.29, 3.21 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.53-2.67, 2.18-2.41 (each 2H, m, 17-CH_2CH_2), 2.08 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.77 (3H, d, *J*=7 Hz, 18-CH_3), 1.68 (3H, t, *J*=7 Hz, 8^1-CH_3). MS (CI) found: *m/z* 629. Calcd for $C_{34}H_{37}N_4O_4{}^{64}Zn$: MH^+, 629.

Zinc Methyl 3^1*S*-Bacteriopheophorbide-*d* (3aS). Retention time was 74 min (6 mmϕ Cosmosil, MeOH / H_2O = 3 / 1, 1.0 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 648 (0.68), 602 (0.10), 556 (0.05), 513 (0.04), 422 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.49, 9.42, 8.36 (each 1H, s, 5-, 10-, 20-H), 6.27 (1H, q, *J*=7 Hz, 3-CH), 5.12, 4.98 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.43 (1H, q, *J*=7 Hz, 18-H), 4.13-4.23 (1H, m, 17-H), 3.70 (2H, q, *J*=7 Hz, 8-CH_2), 3.54, 3.31, 3.28, 3.21 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.52-2.68, 2.18-2.42 (each 2H, m, 17-CH_2CH_2), 2.08 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.76 (3H, d, *J*=7

Hz, 18-CH_3), 1.67 (3H, t, J=7 Hz, 8^1-CH_3). MS (CI) found: *m/z* 629. Calcd for $C_{34}H_{37}N_4O_4{}^{64}Zn$: MH^+, 629.

Zinc Methyl 20-Fluorobacteriopheophorbide-*d* ($3^1R/3^1S$=1/1) (3b). Retention time was 5.8 min (Gelpack, MeOH, 1.5 mL / min); dark green solids; mp > 300 °C; VIS (CH_2Cl_2) λ_{max} = 651.5 (0.50), 606 (0.08), 567 (0.05), 522.5 (0.03), 422 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.89, 9.63 (each 1H, s, 5-, 10-H), 6.36 (1H, q, J=7 Hz, 3-CH), 5.25, 5.16 (1H, d, J=20 Hz, 13^1-CH_2), 4.75 (1H, q, J=7 Hz, 18-H), 4.28-4.37 (each 1H, m, 17-H), 3.81 (2H, q, J=7 Hz, 8-CH_2), 3.64, 3.51/49, 3.31 (each 3H, s, 17^2-CO_2CH_3, 7-, 12-CH_3), 3.400/3.395 (3H, d, J_{HF}=5 Hz, 2-CH_3), 2.51-2.71, 2.19-2.39 (each 2H, m, 17-CH_2CH_2), 2.13 (3H, d, J=7 Hz, 3^1-CH_3), 1.74 (3H, d, J=7 Hz, 18-CH_3), 1.75/73 (3H, t, J=7 Hz, 8^1-CH_3). MS (FAB) found: *m/z* 646. Calcd for $C_{34}H_{35}N_4O_4F^{64}Zn$: M^+, 646.

Zinc Methyl 3^1R-20-Fluorobacteriopheophorbide-*d* (3bR). Retention time was 55 min (6 mmϕ Cosmosil, MeOH / H_2O = 3 / 1, 1.2 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 651.5 (0.49), 606 (0.08), 567.5 (0.06), 522 (0.02), 422 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.89, 9.62 (each 1H, s, 5-, 10-H), 6.36 (1H, q, J=7 Hz, 3-CH), 5.24, 5.15 (1H, d, J=20 Hz, 13^1-CH_2), 4.77 (1H, q, J=7 Hz, 18-H), 4.27-4.35 (each 1H, m, 17-H), 3.80 (2H, q, J=7 Hz, 8-CH_2), 3.64, 3.49, 3.31 (each 3H, s, 17^2-CO_2CH_3, 7-, 12-CH_3), 3.40 (3H, d, J_{HF}=5 Hz, 2-CH_3), 2.53-2.70, 2.18-2.37 (each 2H, m, 17-CH_2CH_2), 2.13 (3H, d, J=7 Hz, 3^1-CH_3), 1.75 (3H, d, J=7 Hz, 18-CH_3), 1.73 (3H, t, J=7 Hz, 8^1-CH_3). MS (FAB) found: *m/z* 646. Calcd for $C_{34}H_{35}N_4O_4F^{64}Zn$: M^+, 646.

Zinc Methyl 3^1S-20-Fluorobacteriopheophorbide-*d* (3bS). Retention time was 68 min (6 mmϕ Cosmosil, MeOH / H_2O = 3 / 1, 1.2 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 651.5 (0.52), 605 (0.09), 567 (0.05), 524 (0.03), 422 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.89, 9.62 (each 1H, s, 5-, 10-H), 6.36 (1H, q, J=7 Hz, 3-CH), 5.24, 5.15 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.78 (1H, q, J=7 Hz, 18-H), 4.28-4.35 (1H, m, 17-H), 3.80 (2H, q, J=7 Hz, 8-CH_2), 3.63, 3.51, 3.31 (each 3H, s, 17^2-CO_2CH_3, 7-, 12-CH_3), 3.39 (3H, d, J_{HF}=5 Hz, 2-CH_3), 2.52-2.71, 2.20-2.37 (each 2H, m, 17-CH_2CH_2), 2.13 (3H, d, J=7 Hz, 3^1-CH_3), 1.74 (3H, d, J=7 Hz, 18-CH_3), 1.72 (3H, t, J=7 Hz, 8^1-CH_3). MS (FAB) found: *m/z* 646. Calcd for $C_{34}H_{35}N_4O_4F^{64}Zn$: M^+, 646.

Methyl 3^1R-20-Fluorobacteriopheophorbide-*d* (2bR). Black solids; VIS (CH_2Cl_2) λ_{max} = 664 (0.39), 605 (0.07), 542 (0.11), 510.5 (0.10), 409.5 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ = 10.03 (1H, s, 5-H), 9.60 (1H, s, 10-H), 6.52 (1H, q, J=7 Hz, 3-CH), 5.32, 5.22 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.77 (1H, q, J=7 Hz, 18-H), 4.30-4.38 (1H, m, 17-H), 3.73 (2H, q, J=7 Hz, 8-CH_2), 3.72 (3H, s, 12-CH_3), 3.59 (3H, s, 17^2-CO_2CH_3), 3.55 (3H, d, J_{HF}=4 Hz, 2-CH_3), 3.32 (3H, s, 7-CH_3), 2.51-2.75, 2.10-2.36 (each 2H, m, 17-CH_2CH_2), 2.20 (3H, d, J=7 Hz, 3^1-CH_3), 1.76 (3H, t, J=7 Hz, 8^1-CH_3), 1.75 (3H, d, J=7 Hz, 18-CH_3), -2.88 (1H, s, NH^{19}). MS (FAB) found: *m/z* 585. Calcd for $C_{34}H_{38}N_4O_4F$: MH^+, 585.

Methyl 3^1S-20-Fluorobacteriopheophorbide-*d* (2bS). Black solids; VIS (CH_2Cl_2) λ_{max} = 665 (0.35), 607.5 (0.06), 543 (0.10), 510 (0.09), 409.5 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ = 10.04 (1H, s, 5-H), 9.60 (1H, s, 10-H), 6.51 (1H, q, J=7 Hz, 3-CH), 5.31, 5.21 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.76 (1H, q, J=7 Hz, 18-H), 4.30-4.38 (1H, m, 17-H), 3.73 (2H, q, J=7 Hz, 8-CH_2), 3.72 (3H, s, 12-CH_3), 3.60 (3H, s, 17^2-CO_2CH_3), 3.56 (3H, d, J_{HF}=4 Hz, 2-CH_3), 3.32 (3H, s, 7-CH_3), 2.51-2.75, 2.10-2.36 (each 2H, m, 17-CH_2CH_2), 2.19 (3H, d, J=7 Hz, 3^1-CH_3), 1.72 (3H, t, J=7 Hz, 8^1-CH_3), 1.70 (3H, d, J=7 Hz, 18-CH_3), -2.89 (1H, s, NH^{19}). MS (FAB) found: *m/z* 584. Calcd for $C_{34}H_{37}N_4O_4F$: M^+, 584 .

Zinc Methyl 20-Chlorobacteriopheophorbide-*d* ($3^1R/3^1S$=1/1) (3c). Retention time was 6.7 min (Gelpack, MeOH, 1.5 mL / min); dark green solids; mp > 300 °C; VIS (CH_2Cl_2) λ_{max} = 657.5 (0.58), 613 (0.10), 573 (0.05), 425 nm (1.00); ^{1}H-NMR (CD_3OD)[22] δ = 9.92/90, 9.57 (each 1H, s, 5-, 10-H), 6.33 (1H,

q, *J*=7 Hz, 3-CH), 5.14, 5.08 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.15-4.19 (1H, m, 17-H), 3.77 (2H, q, *J*=8 Hz, 8-CH_2), 3.59, 3.49/48, 3.46, 3.26 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.39-2.62, 2.22-2.31 (each 2H, m, 17-CH_2CH_2), 2.12/09 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.71 (3H, t, *J*=8 Hz, 8^1-CH_3), 1.62/60 (3H, d, *J*=6 Hz, 18-CH_3). MS (FAB) found: *m/z* 662. Calcd for $C_{34}H_{35}N_4O_4{}^{35}Cl^{64}Zn$: M^+, 662.

Zinc Methyl 3^1*R*-20-Chlorobacteriopheophorbide-*d* (3cR). Retention time was 45 min (6 mmϕ Cosmosil, MeOH / H_2O = 4 / 1, 1.2 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 657 (0.57), 611.5 (0.10), 572.5 (0.07), 425 nm (1.00); ^{1}H-NMR (CD_3OD)[22] δ = 9.90, 9.57 (each 1H, s, 5-, 10-H), 6.33 (1H, q, *J*=7 Hz, 3-CH), 5.14, 5.08 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.15-4.19 (1H, m, 17-H), 3.77 (2H, q, *J*=8 Hz, 8-CH_2), 3.59, 3.49, 3.46, 3.26 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.39-2.62, 2.22-2.31 (each 2H, m, 17-CH_2CH_2), 2.12 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.71 (3H, t, *J*=8 Hz, 8^1-CH_3), 1.62 (3H, d, *J*=6 Hz, 18-CH_3). MS (FAB) found: *m/z* 662. Calcd for $C_{34}H_{35}N_4O_4{}^{35}Cl^{64}Zn$: M^+, 662.

Zinc Methyl 3^1*S*-20-Chlorobacteriopheophorbide-*d* (3cS). Retention time was 53 min (6 mmϕ Cosmosil, MeOH / H_2O = 4 / 1, 1.2 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 656.5 (0.55), 611 (0.09), 571 (0.07), 425 nm (1.00); ^{1}H-NMR (CD_3OD)[22] δ = 9.92, 9.57 (each 1H, s, 5-, 10-H), 6.33 (1H, q, *J*=7 Hz, 3-CH), 5.14, 5.08 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.15-4.19 (1H, m, 17-H), 3.77 (2H, q, *J*=8 Hz, 8-CH_2), 3.59, 3.48, 3.46, 3.26 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.39-2.62, 2.22-2.31 (each 2H, m, 17-CH_2CH_2), 2.09 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.71 (3H, t, *J*=8 Hz, 8^1-CH_3), 1.60 (3H, d, *J*=6 Hz, 18-CH_3). MS (FAB) found: *m/z* 662. Calcd for $C_{34}H_{35}N_4O_4{}^{35}Cl^{64}Zn$: M^+, 662.

Methyl 3^1*R*-20-Chlorobacteriopheophorbide-*d* (2cR). Black solids; VIS (CH_2Cl_2) λ_{max} = 671.5 (0.45), 613 (0.06), 548.5 (0.11), 516 (0.07), 412.5 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ = 10.02 (1H, s, 5-H), 9.42 (1H, s, 10-H), 6.48 (1H, q, *J*=7 Hz, 3-CH), 5.11, 5.02 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.77 (1H, q, *J*=7 Hz, 18-H), 4.05-4.23 (1H, m, 17-H), 3.69 (2H, q, *J*=7 Hz, 8-CH_2), 3.63 (3H, s, 12-CH_3), 3.60 (3H, s, 17^2-CO_2CH_3), 3.59 (3H, s, 2-CH_3), 3.28 (3H, s, 7-CH_3), 2.55-2.67, 2.20-2.45 (each 2H, m, 17-CH_2CH_2), 2.14 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.68 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.57 (3H, d, *J*=7 Hz, 18-CH_3), -2.12 (1H, s, NH^{19}). MS (FAB) found: *m/z* 600. Calcd for $C_{34}H_{37}N_4O_4{}^{35}Cl$: M^+, 600.

Methyl 3^1*S*-20-Chlorobacteriopheophorbide-*d* (2cS). Black solids; VIS (CH_2Cl_2) λ_{max} = 671.5 (0.45), 612 (0.05), 548 (0.11), 516.5 (0.07), 412 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ = 10.07 (1H, s, 5-H), 9.39 (1H, s, 10-H), 6.44 (1H, q, *J*=7 Hz, 3-CH), 5.11, 5.02 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.74 (1H, q, *J*=7 Hz, 18-H), 4.05-4.23 (1H, m, 17-H), 3.69 (2H, q, *J*=7 Hz, 8-CH_2), 3.63 (3H, s, 12-CH_3), 3.59 (3H, s, 17^2-CO_2CH_3), 3.58 (3H, s, 2-CH_3), 3.27 (3H, s, 7-CH_3), 2.55-2.67, 2.20-2.45 (each 2H, m, 17-CH_2CH_2), 2.16 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.68 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.51 (3H, d, *J*=7 Hz, 18-CH_3), -2.12 (1H, s, NH^{19}). MS (FAB) found: *m/z* 600. Calcd for $C_{34}H_{37}N_4O_4{}^{35}Cl$: M^+, 600.

Zinc Methyl Bacteriopheophorbide-*c* (3^1*R*/3^1*S*=2/1) (3d). Retention time was 6.0 min (Gelpack, MeOH, 1.5 mL / min); dark green solids; mp > 300 °C; VIS (CH_2Cl_2) λ_{max} = 658 (0.61), 612 (0.10), 574 (0.06), 427 nm (1.00); ^{1}H-NMR (CD_3OD) δ (*R*/*S*) = 9.73/71, 9.42 (each 1H, s, 5-, 10-H), 6.32 (1H, q, *J*=7 Hz, 3-CH), 5.06, 5.03 (each 1H, d, *J*=20 Hz, 13^1-CH_2), 4.53 (1H, q, *J*=7 Hz, 18-H), 4.03 (1H, dd, *J*=3, 8 Hz, 17-H), 3.74 (3H, s, 20-CH_3), 3.70 (2H, q, *J*=8 Hz, 8-CH_2), 3.53, 3.46/47, 3.33, 3.23 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.42-2.59, 2.24-2.35, 1.98-2.22 (1H+1H+2H, m, 17-CH_2CH_2), 2.07/09 (3H, d, *J*=7 Hz, 3^1-CH_3), 1.68 (3H, t, *J*=8 Hz, 8^1-CH_3), 1.44/43 (3H, d, *J*=7 Hz, 18-CH_3). MS (FAB) found: *m/z* 642. Calcd for $C_{35}H_{38}N_4O_4{}^{64}Zn$: M^+, 642.

Zinc Methyl 3^1*R*-Bacteriopheophorbide-*c* (3dR). Retention time was 24 min (6 mmϕ Cosmosil, MeOH / H_2O = 4 / 1, 1.5 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 658 (0.63), 612 (0.10), 574 (0.06), 427 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.67, 9.45 (each 1H, s, 5-, 10-H), 6.31 (1H, q, *J*=7 Hz, 3-

CH), 5.14, 5.11 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.53 (1H, q, J=7 Hz, 18-H), 4.05 (1H, m, 17-H), 3.77 (3H, s, 20-CH_3), 3.71 (2H, q, J=8 Hz, 8-CH_2), 3.60, 3.48, 3.33, 3.23 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.33-2.54, 2.09-2.22 (each 2H, m, 17-CH_2CH_2), 2.06 (3H, d, J=7 Hz, 3^1-CH_3), 1.67 (3H, t, J=8 Hz, 8^1-CH_3), 1.50 (3H, d, J=7 Hz, 18-CH_3). MS (FAB) found: m/z 642. Calcd for $C_{35}H_{38}N_4O_4{}^{64}Zn$: M^+, 642.

Zinc Methyl 3^1S-Bacteriopheophorbide-*c* (3dS). Retention time was 27 min (6 mmϕ Cosmosil, MeOH / H_2O = 4 / 1, 1.5 mL / min); dark green solids; VIS (CH_2Cl_2) λ_{max} = 658 (0.61), 612 (0.10), 575 (0.04), 426 nm (1.00); ^{1}H-NMR (CD_3OD) δ = 9.66, 9.43 (each 1H, s, 5-, 10-H), 6.30 (1H, q, J=7 Hz, 3-CH), 5.13, 5.10 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.50 (1H, q, J=7 Hz, 18-H), 4.03 (1H, m, 17-H), 3.75 (3H, s, 20-CH_3), 3.66 (2H, q, J=8 Hz, 8-CH_2), 3.59, 3.47, 3.31, 3.21 (each 3H, s, 17^2-CO_2CH_3, 2-, 7-, 12-CH_3), 2.37-2.55, 2.10-2.27 (each 2H, m, 17-CH_2CH_2), 2.07 (3H, d, J=7 Hz, 3^1-CH_3), 1.64 (3H, t, J=8 Hz, 8^1-CH_3), 1.44 (3H, d, J=7 Hz, 18-CH_3). MS (FAB) found: m/z 642. Calcd for $C_{35}H_{38}N_4O_4{}^{64}Zn$: M^+, 642.

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