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Self-aggregation of synthetic zinc chlorophyll derivative possessing a perfluoroalkyl group in a fluorinated solvent

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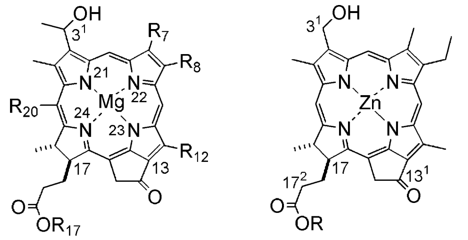
Abstract—Zinc 3^1-hydroxy-13^1-oxo-chlorin possessing a perfluoroheptyl group on the 17-propionate was prepared by modifying chlorophyll *a*. The synthetic compound self-aggregated in 0.1% (v/v) THF and HCFC225cb ($CClF_2CF_2CHClF$) to give similar large oligomers to natural light-harvesting antennae of green photosynthetic bacteria and their models. Visible absorption, circular dichroism, and fluorescence emission spectra showed that F–F interaction between the perfluoroheptyl side chain and the fluorinated solvent (HCFC225cb) stabilized the supramolecular structure. The core part of the supramolecular structure was constructed by a special bond of $Zn\cdots O3^1\text{-}H\cdots O{=}C13^1$ and π–π stacking, and the 17-propionates were at the peripheral part.

1. Introduction

Generally light-harvesting antennae of photosynthetic organisms are complexes of pigments (chlorophylls, mainly) with proteins.[1] On the other hand, green photosynthetic bacteria possess unique major antennae, so-called chlorosomes, where self-aggregates of bacteriochlorophylls (BChl) *c*, *d*, and *e* (Fig. 1, left) are covered with a lipid monolayer.[2–12] Sunlight energy is first absorbed at the large oligomer of these BChls in an extramembranous chlorosome, then transferred efficiently and rapidly to a reaction center in a membrane through various complexes of BChls-*a* with proteins.[8–12] In a chlorosome, the composite BChl molecules specifically interact with each other through the 3^1-hydroxy, 13-keto carbonyl groups and the central magnesium on the Q_y axis (N21–N23) to form large self-aggregates.[13] In contrast, these BChls have a variety of substituents at R_7, R_8 and in R_{17} on the Q_x axis (N22–N24) as shown in Figure 1. Many investigations of the R_7 and R_8 groups have been reported,[14] but few studies are available for the R_{17} group.[15–20] In naturally occurring BChls-*c*, *d*, and *e*, some long hydrocarbon chains at the 17-propionate were found, including farnesyl, stearyl, phytyl, and others.[1,21]



BChl-*c* : R_7 = Me, R_{20} = Me
BChl-*d* : R_7 = Me, R_{20} = H
BChl-*e* : R_7 = CHO, R_{20} = Me
R_8 = Et, *n*Pr, *i*Bu, *neo*Pen
R_{12} = Me, Et
R_{17} = farnesyl, stearyl, phytyl, etc.

1 : R = $(CH_2)_3NHCO(CF_2)_7F$
2 : R = $(CH_2)_3NHCO(CH_2)_7H$

Figure 1. Molecular structures of BChls-*c*, *d*, *e* (left) and their synthetic zinc chlorin models **1**, **2** (right).

Their roles in the self-aggregates have been claimed to be stabilization of the supramolecular structure by interactions among hydrocarbon chains of BChls as well as among those of BChls and surrounding lipids,[16,20,22] but has still not been clearly identified.

Synthetic zinc 3^1-hydroxy-13^1-oxo-chlorins have been used as model compounds of BChls-*c*, *d*, and *e* due to their availability and stability.[2,23] They have self-aggregated in many environments including non-polar organic solvents and aqueous solutions to form similar oligomers to natural chlorosomal self-aggregates. Here we synthesized zinc

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Keywords: Fluorous chemistry; Light-harvesting complex; Photosynthetic antenna; Supramolecule.

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chlorophylls possessing a perfluoroalkyl chain in the R_{17}-group (Fig. 1, right) and examined its self-aggregation in a fluorinated solvent. We also comment on the function of a long hydrocarbon chain (R_{17}) in chlorosomal self-aggregates based on the present study.

2. Results and discussion

2.1. Synthesis of fluorinated zinc chlorin possessing 3^1-hydroxy and 13^1-oxo-groups

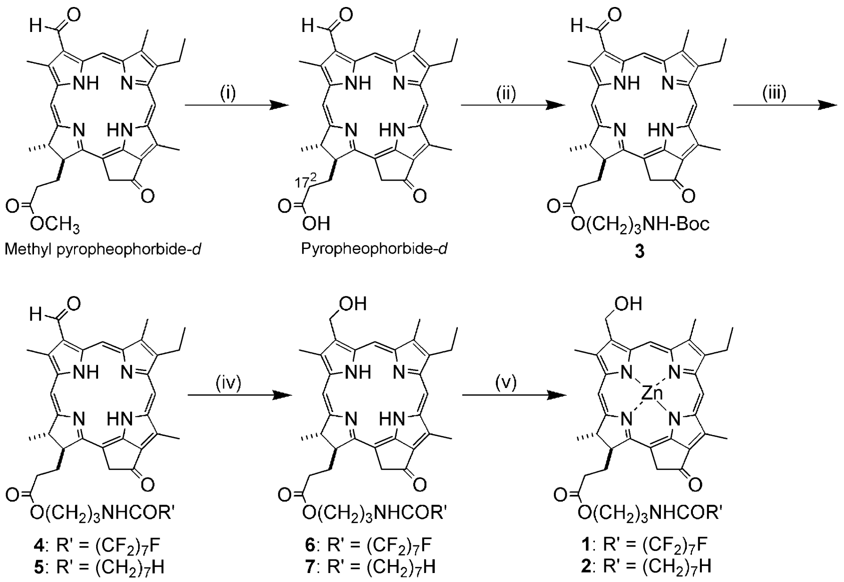
We first esterified the 17^2-carboxy group of chlorophyll molecules by fluorinated alcohols $RfCH_2OH$ and $RfCH_2CH_2OH$ (Rf means a perfluoroalkyl group). Metal-free chlorins possessing 17^2-$COOCH_2Rf$ could be obtained but were easily trans-esterified with an additional alcohol by the influence of electronegativity of the Rf-group. As solvents for zinc-metallation and purification of zinc complexes, alcohols (usually methanol) were necessary and zinc complexes having the 17^2-$COOCH_2Rf$ could not be isolated in their pure form: a mixture of the desired product and the corresponding trans-esterified by-product. On the other hand, desired zinc complexes possessing a CH_2CH_2 spacer (17^2-$COOCH_2CH_2Rf$) were synthesized but the pure samples were difficult to handle due to their low solubility in ordinary organic solvents. It is noteworthy that the esterification was performed in at most 50% yield because $RfCH_2CH_2OH$ had a relatively low solubility in a solvent for esterification (usually dry CH_2Cl_2).

To overcome this lower solubility, we next used fluorinated carboxylic acid halides to introduce perfluoroalkyl groups at the 17-propionate. A series of fluorinated acid halides (RfCOX) were commercially available, and were more soluble and reactive than the corresponding alcohol, $Rf(CH_2)_nOH$. Esterification of 17^2-$COOCH_2$-CH_2OH[18] with RfCOCl occurred smoothly (∼80% yield) as expected, but the resulting $RfCOOCH_2$—was cleaved during the successive reduction of 3-CHO → 3-CH_2OH to give $RfCH_2OH$ and $HOCH_2$—due to the electronegativity of Rf-group. Therefore, an amido linkage was selected for preparing desired compounds ($R = CH_2CH_2CH_2NHCORf$). Amidation of 17^2-$COO(CH_2)_3NH_2$ with RfCOCl proceeded efficiently ($\geqslant$70% yield) and the resulting compounds were further modified without altering the RfCONH-group. Synthetic procedures of zinc 3^1-hydroxy-13^1-oxo-chlorin **1** possessing a perfluoroheptyl group in the 17-propionate are described below.

The perfluoroalkyl group was introduced into chlorins as follows (Scheme 1). Pyropheophorbide-*d* was obtained by treating methyl pyropheophorbide-*d* in concd HCl.[24] The 17^2-carboxyl group of pyropheophorbide-*d* was esterified with *N*-Boc-protected β-alaninol by using 1-{3-(*N*,*N*-dimethylamino)propyl}-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP).[18] The Boc group of **3** was selectively deprotected under an acidic condition and the resulting amine was reacted with perfluorooctanoyl chloride to give the corresponding amide **4** possessing a perfluoroheptyl group on the 17-position. The 3-formyl group of **4** was selectively reduced into the 3-hydroxymethyl group of **6** followed by zinc metallation[16,23] to give the corresponding zinc chlorin **1**. Zinc chlorin **2** possessing a heptyl group on the 17-position was synthesized similarly using octanoyl chloride. All the synthetic compounds **1**–**7** were identified by their visible, ^1H NMR, and MS spectra and zinc complexes, **1** and **2**, were purified by reversed-phase high-performance liquid chromatography (HPLC).

2.2. Fluorinated zinc chlorin in non-polar organic and flon solvents

Both zinc chlorins **1** and **2** possessing perfluoroheptyl and heptyl groups, respectively, gave the same absorp-



Scheme 1. Synthesis of zinc chlorins possessing a perfluoroheptyl group **1** and a heptyl group **2**. Reagents: (i) concd HCl (ii) $HO(CH_2)_3NH$-Boc, EDC·HCl, DMAP/CH_2Cl_2, (iii) 4 N HCl/AcOEt, $F(CF_2)_7COCl$ or $H(CH_2)_7COCl/CHCl_3$, (iv) $t BuNH_2{\cdot}BH_3/CH_2Cl_2$, and (v) $Zn(OAc)_2{\cdot}2H_2O$/MeOH-$CHCl_3$.

tion maxima of sharp and intense Q_y/Soret bands at 646/424 nm in tetrahydrofuran (THF) (ca. 4 μM, Fig. 2A, upper). They showed identical small circular dichroism (CD) bands at their Q_y/Soret regions in THF (Fig. 2A, lower). These visible and CD spectra indicated that they were monomeric in THF compared with reported data,[23] and also that perfluorination of heptyl group at the 17-propionate did not affect any optical properties of monomeric zinc chlorins. When the THF solutions were diluted with hexane (THF/hexane = 0.4/99.6 (v/v), ca. 1 μM), the Q_y/Soret absorption bands were broadened and red-shifted from 646/424 to about 740/450 nm (Fig. 2B, upper), which were similar to natural chlorosomes and their artificial models.[15,23] These spectral changes were ascribable to self-aggregates of **1** and **2** through $\text{Zn}\cdots\text{O3}^1\text{-H}\cdots\text{O=C13}^1$ and π–π interaction.[25] Q_y maximum of self-aggregated **1** was measured at 738 nm and the value was 4-nm smaller than that in $(\mathbf{2})_n$ (742 nm). Fluorescence emission peaks from self-aggregates of **1** and **2** (excitation at 447 nm) were observed at 744 and 747 nm, respectively, which is consistent with the visible spectral change. The small but obvious differences showed that the heptyl group would form slightly more stabilized self-aggregates of **2** in the hexane based solvent than the perfluoroheptyl group in **1**. On the other hand, the two CD spectra were almost the same (Fig. 2B, lower) and major parts of their supramolecules would be similar to the natural and artificial chlorosome.[16]

We chose 1,3-dichloro-1,1,2,2,3-pentafluoropropane (HCFC225cb, $\text{CClF}_2\text{CF}_2\text{CHClF}$), one of the partially fluorinated solvents, as a fluorinated solvent. This solvent is penta-fluorinated propane (5/8 fluorination) and has both fluorophilic and lipophilic properties. Thus, HCFC225cb easily dissolves most fluorinated compounds and mixes almost organic solvents in any proportion. Neither of the present compounds **1**[26] and **2** could be dissolved solely in HCFC225cb and their optical spectra were measured in 0.1% (v/v) THF and HCFC225cb (ca. 6 μM, Fig. 3). In a 1000-fold dilution of THF solutions of **1** and **2** with HCFC225cb appeared new broadened and red-shifted Q_y/Soret bands at around 740/450 nm (Fig. 3, upper). Both **1** and **2** also self-aggregated in the fluorinated solvent to form similar large oligomers. The Q_y peak position of **1** was 741 nm and moved to a 4-nm longer wavelength than that of **2** (737 nm). In fluorescence emission spectra excited at 450 nm, a similar shift was observed: 748 for $(\mathbf{2})_n \rightarrow 750$ nm for $(\mathbf{1})_n$. These shifts were just the reverse of those in a hexane based solvent, indicating that self-aggregates of **1** possessing a perfluoroheptyl group in HCFC225cb should be more stabilized than those of **2**. In 0.1% (v/v) THF and HCFC225cb, **1** gave pronounced reverse S-shaped CD bands at the red-shifted Q_y region (solid line of Fig. 3, lower), while **2** showed a mixture of reverse S- and S-shaped bands (dotted line of Fig. 3, lower). These CD observations indicated that **1** would form ordered self-aggregates in the fluorinated solvent than **2**, compared with previous reports.[16]

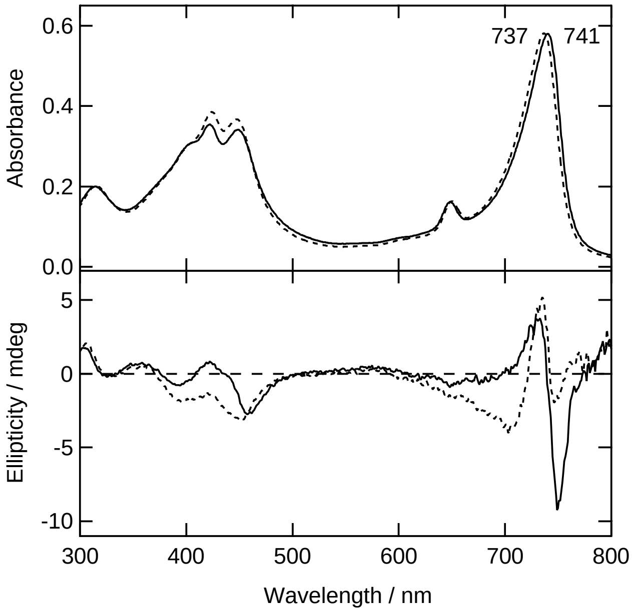


Figure 3. Visible absorption (upper) and CD spectra (lower) of **1** (solid line) and **2** (dotted line) in 0.1% (v/v) THF-HCFC225cb (ca. 6 μM).

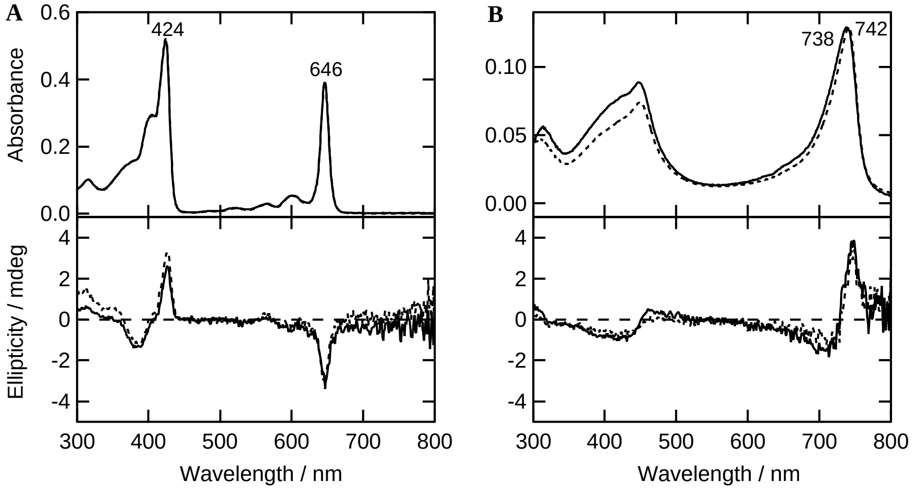


Figure 2. Visible absorption (upper) and CD spectra (lower) of **1** (solid line) and **2** (dotted line) in THF (A, ca. 4 μM) and 0.4% (v/v) THF–hexane (B, ca. 1 μM).

From the above optical spectral analyses, the following points were claimed. Both **1** and **2** self-aggregated in the hexane and HCFC225cb based solvents to form similar oligomers irrespective of fluorination at the 17-propionate. Self-aggregation in HCFC225cb was first reported, while hexane is well known to be efficient for the self-aggregation. Interaction of the heptyl group at the 17-propionate with hexane as a solvent would relatively stabilize self-aggregates of **2** and interaction of the fluoroheptyl group with HCFC225cb would reversely stabilize (**1**)$_n$, although major supramolecular structures of all the self-aggregates were similar ($\mathrm{Zn}\cdots\mathrm{O3^1\text{-}H}\cdots\mathrm{O{=}C13^1}/\pi\text{–}\pi$ interaction). It is noteworthy that no amido linkage on the substituent disturbed the formation of self-aggregates as reported earlier.[17]

2.3. Deaggregation of self-aggregates in HCFC225cb

In 0.1% (v/v) THF and HCFC225cb, small monomeric Q$_y$ bands of **1** and **2** (ca. 6 μM) were observed at 650 nm (Fig. 3, upper). The monomeric species still remained at 10% (mole percentage based on monomer concentration) even in >5 μM solutions. When the concentration decreased (<5 μM), the self-aggregated peak at around 740 nm gradually decreased and concomitantly the monomeric peak at 650 nm increased. Self-aggregation of **1** and **2** was disturbed when their concentration decreased. At 1.4 μM, **1** showed an apparent self-aggregated peak but **2** gave faint self-aggregated and almost monomeric peaks (Fig. 4). The result also indicated stabilized self-aggregates of **1** in a HCFC225cb solution compared with those of **2**.

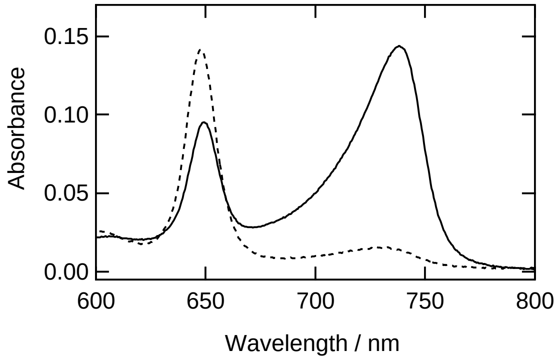


Figure 4. Visible absorption spectra of **1** (solid line) and **2** (dotted line) in 0.1% (v/v) THF-HCFC225cb (ca. 1.4 μM).

Both self-aggregates of **1** and **2** in 0.1% (v/v) THF and HCFC225cb (ca. 6 μM) were dissociated by addition of pyridine to give the corresponding monomeric forms. Visible spectral changes as shown in Figures 5A and B indicated the deaggregation was quite a simple and clean process due to the observation of some isosbestic points. From Figures 5C and D, the Q$_y$ band of self-aggregated **1** was halved by 0.3 μl addition of pyridine, which was the same on halvation in (**2**)$_n$. No difference was observed in deaggregation of (**1**)$_n$ and (**2**)$_n$ by addition of pyridine. This is ascribed to the fact that a strongly basic pyridine directly broke the coordination bond of central Zn with 3^1-OH in the core part of self-aggregates and was insensitive to peripheral interaction of the perfluoroheptyl group with HCFC225cb. The same deaggregation in self-aggregates of **1** and **2** showed that both their main supramolecular structures were constructed with the same motifs: a special bond of $\mathrm{Zn}\cdots\mathrm{O3^1\text{-}H}\cdots\mathrm{O{=}C13^1}$ and π–π stacking as reported.[2,23,27]

Visible absorption spectra of **1** as well as **2** in 0.1% (v/v) THF and HCFC225cb (ca. 6 μM) were not changed at all by cooling (25 → 5 °C) or heating (25 → 40 °C). The remaining monomers could not form self-aggre-

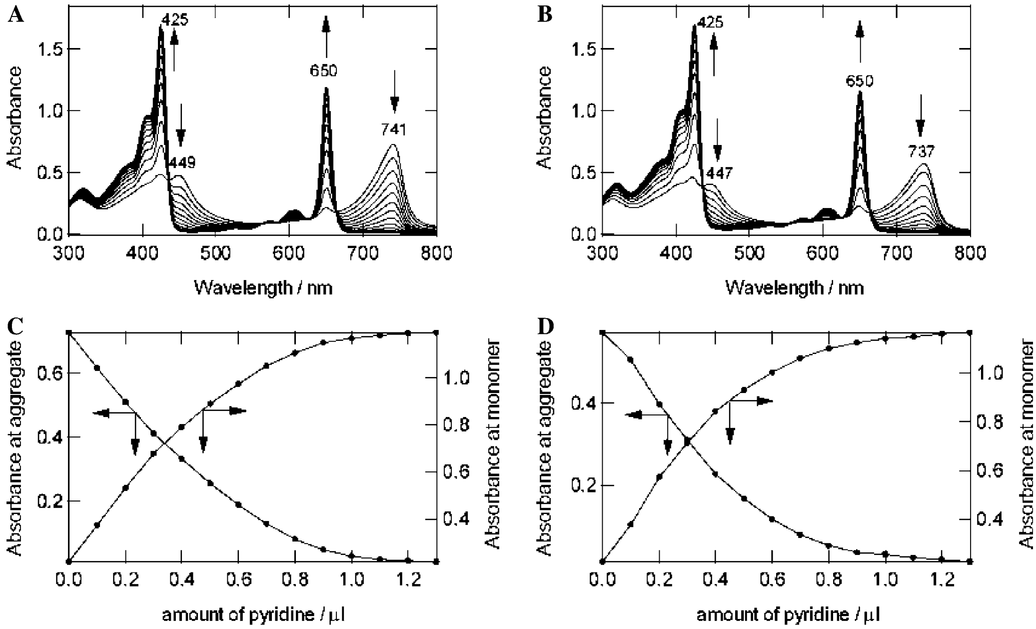


Figure 5. Visible absorption spectral change of **1** (A) and **2** (B) in 0.1% (v/v) THF-HCFC225cb by addition of pyridine and dependency of Q$_y$ peak absorbances of **1** (C) and **2** (D) in 2 ml solution with additional pyridine.

gates by cooling and the formed self-aggregates could not dissociate to monomeric forms by heating. The insensitivity of self-aggregates by thermal changes indicated that the self-aggregation of **1** and **2** was not based on any equilibrium processes. Such a disequilibrium was supported by the fact that about 10% of monomeric species were observed at 5–10 μM concentration, as mentioned above.

Moreover, the disequilibrium for the self-aggregates did not permit estimation of aggregate numbers from concentration dependent spectral analysis. Using a conventional log–log plot (self-aggregated Q_y absorbance vs monomeric Q_y absorbance),[28] both the slopes for **1** and **2** were nonlinear, also indicating the disequilibrium of the present self-aggregation.[29,30]

3. Conclusion

We efficiently synthesized zinc 3^1-hydroxy-13^1-oxo-chlorins possessing a perfluoroheptyl (**1**) and heptyl groups (**2**) on the 17-propionate as stable model compounds of chlorosomal chlorophylls. These compounds self-aggregated in 0.1% (v/v) THF and HCFC225cb as well as 1% (v/v) THF and hexane to give large oligomers with broadened and red-shifted Q_y bands (see Table 1). This is ascribable to the formation of similar supramolecular structures based on a specific $\mathrm{Zn}\cdots\mathrm{O3^1\text{-}H}\cdots\mathrm{O{=}C13^1}$ bonding along their Q_y axes. The Q_y peak position of **1** moved to a 4-nm longer wavelength than that of **2** in the flon solution, while that of **1** moved to a 4-nm shorter wavelength than that of **2** in the hexane solution. These indicated stabilizing self-aggregates of **1** by interaction of the perfluoroheptyl group with HCFC225cb and also that of **2** by interaction of the heptyl group with hexane. Such stabilization of self-aggregates through interaction of the 17-propionate on the Q_x axis with solvents showed that the 17-propionate occupied the peripheral parts of self-aggregated supramolecules.

4. Experimental

4.1. Apparatus

Visible absorption and fluorescence emission spectra were measured with a Hitachi U-3500 spectrometer and F-4500 fluorescence spectrophotometer, respectively. CD spectra were observed with a Jasco J-720W spectropolarimeter. ^1H and ^{19}F NMR spectra were measured with a JEOL ECA-600 spectrometer; chemical shifts (δs) are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) as an internal reference and CF_3COOH (−77 ppm) as an external reference. Mass spectra were recorded on a JEOL Gcmate II spectrometer; fast atomic bombardment (FAB)-MS samples were dissolved in CH_2Cl_2 or THF and *m*-nitrobenzyl alcohol was used as a matrix. HPLC was performed with a Shimadzu LC-10ADVP pump, SPD-M10AVP diode-array detector, and SCL-10AVP system controller.

Table 1. Q_y absorption spectral data in monomeric and oligomeric **1** and **2**

	$\lambda_{\max}$[a]		Δ[b]	$\lambda_{\max}$[a]	Δ[b]
	THF	0.4% (v/v) THF-hexane		0.1% (v/v) THF-HCFC225cb	
1	646	738	1930	741	1980
2	646	742	2000	737	1910

[a] Q_y maximum (nm).

[b] Red-shift (cm^{-1}) of $\lambda_{\max}$ by self-aggregation [= $\{1/\lambda_{\max}(\text{monomer}) - 1/\lambda_{\max}(\text{oligomer})\} \times 10^7$].

4.2. Materials

Solvents in esterification and for measurements of visible and CD spectra were freshly distilled over CaH_2 before use. Flash chromatography (FCC) was carried out on silica gel (Merck Kieselgel 60, 9358). Methyl pyropheophorbide-*d* was prepared according to reported procedures.[16,23] All synthetic procedures were done in the dark. HCFC225cb (AK-225G) was purchased from Asahi Glass Co., Ltd.

4.2.1. Esterification of the 17^2-carboxyl group. A solution of methyl pyropheophorbide-*d* (41.0 mg, 75 μmol) in concd HCl (20 ml) was stirred for 2 h at room temperature. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The aqueous phase was re-extracted with $CHCl_3$ twice and the combined $CHCl_3$ phases were washed with aq 4% $KHSO_4$ and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from CH_2Cl_2 and hexane to give the corresponding carboxylic acid, pyropheophorbide-*d*.

To a dry CH_2Cl_2 solution (20 ml) of the above acid (21.8 mg, 41 μmol), an appropriate alcohol (10 equiv), EDC·HCl (1.5 equiv), and DMAP (2.9 equiv) were added at 0 °C and stirred overnight at room temperature. The reaction mixture was washed with aq 2% HCl, aq 4% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by FCC (Et_2O and CH_2Cl_2) and recrystallization (CH_2Cl_2 and hexane) to give the corresponding ester, pyropheophorbide-*d* ester.

4.2.2. Amidation. A solution of Boc-protected pyropheophorbide-*d* ester **3** (24.1 mg, 35 μmol) in 4 N HCl/AcOEt (10 ml) was stirred at room temperature. After stirring for 2 h, the solution was poured into H_2O at 0 °C and the aqueous layer was extracted with several portions of $CHCl_3$. The combined organic phases were washed with aq 4% $NaHCO_3$ and H_2O, and dried over Na_2SO_4. The resulting solution was then cooled at 0 °C and a carboxylic chloride (1.5 equiv) was added with stirring for 15 min. The mixture was poured into H_2O and the aqueous layer was extracted with several portions of $CHCl_3$. The combined organic phases were washed with aq 4% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by FCC (Et_2O and CH_2Cl_2) and recrystallization (CH_2Cl_2 and hexane) to give the corresponding amide.

4.2.3. Reduction of the 3-formyl group. Borane *t*-butylamine complex (1.5 equiv) was added to a solution of pyropheophorbide-*d* ester (21 μmol) in CH_2Cl_2 (5 ml) at room temperature. After stirring for 2 h, the solution was poured into H_2O at room temperature and the aqueous layer was extracted with several portions of CH_2Cl_2. The combined organic phases were washed with aq 2% HCl, aq 4% $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by FCC (Et_2O or MeOH and CH_2Cl_2) and recrystallization (CH_2Cl_2 and hexane) to give the corresponding 3-hydroxymethyl-chlorin.

4.2.4. Zinc metallation of a chlorin. A methanol solution saturated with $Zn(OAc)_2 \cdot 2H_2O$ (0.3 ml) was added to a solution of a metal-free chlorin (15 μmol) in $CHCl_3$ (5 ml) and methanol (0.2 ml). After stirring for 2 h, the solution was washed with several portions of aq 4% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by HPLC (methanol) to give the corresponding zinc chlorin.

4.2.5. 3-(*t*-Butoxycarbonyl)amino-1-propyl pyropheophorbide-*d* (3). Dark brown solid, 88% yield (FCC, 10% Et_2O-CH_2Cl_2). Vis (CH_2Cl_2) λ_{max} = 694 (relative intensity, 0.80), 632 (0.09), 554 (0.16), 521 (0.14), 428 (1.0), 387 nm (0.83); ^1H NMR ($CDCl_3$) δ = 11.5 (1H, s, 3-CHO), 10.3 (1H, s, 5-H), 9.62 (1H, s, 10-H), 8.85 (1H, s, 20-H), 5.33, 5.21 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.57 (1H, q, J = 7 Hz, 18-H), 4.52 (1H, br s, NHCO), 4.40 (1H, d, J = 8 Hz, 17-H), 4.06–3.95 (2H, m, $COOCH_2$), 3.78 (3H, s, 2-CH_3), 3.72 (3H, s, 12-CH_3), 3.33 (3H, s, 7-CH_3), 3.73 (2H, q, J = 7 Hz, 8-CH_2), 3.00 (2H, q, J = 6 Hz, $COOC_2CH_2$), 2.74–2.54, 2.35–2.22 (each 2H, m, 17-CH_2CH_2), 1.85 (3H, d, J = 7 Hz, 18-CH_3), 1.72 (3H, t, J = 7 Hz, 8^1-CH_3), 1.65–1.58 (2H, m, $COOCCH_2$), 1.36 (9H, s, $C(CH_3)_3$) −0.11, −2.04 (each 1H, s, NH). MS (FAB) found: *m*/*z* 694. Calcd for $C_{40}H_{48}N_5O_6$: MH^+, 694.

4.2.6. 3-(Perfluorooctanoyl)amino-1-propyl pyropheophorbide-*d* (4). Dark brown solid, 73% yield (FCC, 5–7% Et_2O-CH_2Cl_2). Vis (CH_2Cl_2) λ_{max} = 694 (rel, 0.79), 632 (0.08), 554 (0.15), 521 (0.13), 428 (1.0), 388 nm (0.84); ^1H NMR ($CDCl_3$) δ = 11.5 (1H, s, 3-CHO), 10.4 (1H, s, 5-H), 9.69 (1H, s, 10-H), 8.90 (1H, s, 20-H), 6.76 (1H, br s, NHCO), 5.36, 5.23 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.60 (1H, q, J = 7 Hz, 18-H), 4.44 (1H, d, J = 7 Hz, 17-H), 3.98-3.90 (2H, m, $COOCH_2$), 3.79 (3H, s, 2-CH_3), 3.74 (3H, s, 12-CH_3), 3.35 (3H, s, 7-CH_3), 3.76 (2H, q, J = 7 Hz, 8-CH_2), 3.13 (2H, q, J = 6 Hz, $COOC_2CH_2$), 2.71–2.54, 2.42–2.24 (each 2H, m, 17-CH_2CH_2), 1.87 (3H, d, J = 7 Hz, 18-CH_3), 1.73 (3H, t, J = 7 Hz, 8^1-CH_3), 1.67–1.63 (2H, m, $COOCCH_2$), −0.05, −2.04 (each 1H, s, NH); ^{19}F NMR ($CDCl_3$) δ = −82.0 (3F, CF_3) −121.0, −122.8, −123.2, −123.8, −124.0, −127.4 (each 2F, CF_2). MS (FAB) found: *m*/*z* 990. Calcd for $C_{43}H_{39}F_{15}N_5O_5$: MH^+, 990.

4.2.7. 3-(Octanoyl)amino-1-propyl pyropheophorbide-*d* (5). Dark brown solid, 78% yield (FCC, 0.2% MeOH-$CHCl_3$). Vis (CH_2Cl_2) λ_{max} = 694 (rel, 0.77), 633 (0.08), 554 (0.16), 521 (0.14), 428 (1.0), 388 nm (0.85); ^1H NMR ($CDCl_3$) δ = 11.5 (1H, s, 3-CHO), 10.3 (1H, s, 5-H), 9.60 (1H, s, 10-H), 8.84 (1H, s, 20-H), 5.53 (1H, br s, NHCO), 5.33, 5.21 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.57 (1H, q, J = 7 Hz, 18-H), 4.40 (1H, d, J = 8 Hz, 17-H), 4.00–3.95 (2H, m, $COOCH_2$), 3.78 (3H, s, 2-CH_3), 3.72 (3H, s, 12-CH_3), 3.31 (3H, s, 7-CH_3), 3.73 (2H, q, J = 7 Hz, 8-CH_2), 3.09–2.98 (2H, m, $COOC_2CH_2$), 2.72–2.54, 2.38–2.24 (each 2H, m, 17-CH_2CH_2), 2.04 (2H, t, J = 7 Hz, $NCOCH_2$), 1.85 (3H, d, J = 7 Hz, 18-CH_3), 1.71 (3H, t, J = 7 Hz, 8-CH_3), 1.60–1.56 (2H, m, $COOCCH_2$), 1.54–1.49 (2H, m, $NCOCCH_2$), 1.25–1.18 (8H, m, $NCOC_2(CH_2)_4$), 0.83 (3H, t, J = 7 Hz, $NCOC_6CH_3$), −0.10, −2.05 (each 1H, s, NH). MS (FAB) found: *m*/*z* 720. Calcd for $C_{43}H_{54}N_5O_5$: MH^+, 720.

4.2.8. 3-(Perfluorooctanoyl)amino-1-propyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (6). Black solid, 69% yield (FCC, 2% MeOH-CH_2Cl_2). Vis (CH_2Cl_2) λ_{max} = 662 (rel, 0.46), 605 (0.07), 536 (0.08), 505 (0.09), 410 (1.0), 318 nm (0.19); ^1H NMR ($CDCl_3$) δ = 9.57 (1H, s, 10-H), 9.51 (1H, s, 5-H), 8.62 (1H, s, 20-H), 6.76 (1H, br s, NHCO), 5.90 (2H, s, 3-CH_2), 5.25, 5.13 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.51 (1H, q, J = 7 Hz, 18-H), 4.34 (1H, d, J = 8 Hz, 17-H), 3.91–3.78 (2H, m, $COOCH_2$), 3.67 (3H, s, 12-CH_3), 3.42 (3H, s, 2-CH_3), 3.27 (3H, s, 7-CH_3), 3.72 (2H, q, J = 7 Hz, 8-CH_2), 2.97 (2H, q, J = 6 Hz, $COOC_2CH_2$), 2.66–2.44, 2.21–2.17 (each 2H, m, 17-CH_2CH_2), 1.83 (3H, d, J = 7 Hz, 18-CH_3), 1.70 (3H, t, J = 7 Hz, 8^1-CH_3), 1.64–1.60 (2H, m, $COOCCH_2$), 0.36, −1.82 (each 1H, s, NH); ^{19}F NMR ($CDCl_3$) δ = −82.0 (3F, CF_3) −121.1, −122.8, −123.2, −123.8, −124.0, −127.4 (each 2F, CF_2). MS (FAB) found: *m*/*z* 991. Calcd for $C_{43}H_{40}F_{15}N_5O_5$: M^+, 991.

4.2.9. 3-(Octanoyl)amino-1-propyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (7). Black solid, 47% yield (FCC, 0.5% MeOH-$CHCl_3$). Vis (CH_2Cl_2) λ_{max} = 661 (rel, 0.48), 605 (0.08), 535 (0.09), 504 (0.10), 410 (1.0), 317 nm (0.20); ^1H NMR ($CDCl_3$) δ = 9.46 (1H, s, 10-H), 9.43 (1H, s, 5-H), 8.54 (1H, s, 20-H), 5.89 (2H, s, 3-CH_2), 5.40 (1H, br s, NHCO), 5.18, 5.08 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.46 (1H, q, J = 7 Hz, 18-H), 4.28 (1H, d, J = 8 Hz, 17-H), 3.89–3.77 (2H, m, $COOCH_2$), 3.68 (2H, q, J = 7 Hz, 8-CH_2), 3.64 (3H, s, 12-CH_3), 3.41 (3H, s, 2-CH_3), 3.25 (3H, s, 7-CH_3), 2.88–2.71 (2H, m, $COOC_2CH_2$), 2.63–2.49, 2.40–2.17 (each 2H, m, 17-CH_2CH_2), 1.91 (2H, t, J = 7 Hz, $NCOCH_2$), 1.78 (3H, d, J = 7 Hz, 18-CH_3), 1.68 (3H, t, J = 7 Hz, 8-CH_3), 1.46–1.42 (2H, m, $COOCCH_2$), 1.40–1.37 (2H, m, $NCOCCH_2$), 1.25–1.16 (8H, m, $NCOC_2(CH_2)_4$), 0.82 (3H, t, J = 7 Hz, $NCOC_6CH_3$), 0.30, −1.80 (each 1H, s, NH). MS (FAB) found: *m*/*z* 722. Calcd for $C_{43}H_{56}N_5O_5$: MH^+, 722.

4.2.10. Zinc 3-(perfluorooctanoyl)amino-1-propyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (1). Dark green solid (HPLC, Cosmosil 5C18-AR-II, Nacalai Tesque, 10 × 250 mm with MeOH 1.0 ml/min, the retention time was 17.4 min). Vis (THF) λ_{max} = 646 (rel, 0.76), 601 (0.11), 566 (0.06), 521 (0.03), 424 (1.0), 404 nm (0.57); HRMS (FAB) found: *m*/*z* 1054.2016. Calcd for $C_{43}H_{39}F_{15}N_5O_5$ ^{64}Zn: MH^+, 1054.2005.

4.2.11. Zinc 3-(octanoyl)amino-1-propyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (2). Dark green solid (HPLC, Cosmosil 5C18-AR-II, 10 × 250 mm with

MeOH 1.0 ml/min, the retention time was 16.7 min). Vis (THF) λ_{max} = 646 (rel, 0.76), 599 (0.11), 566 (0.06), 520 (0.04), 424 (1.0), 403 nm (0.56); HRMS (FAB) found: *m*/*z* 783.3376. Calcd for $C_{43}H_{53}N_5O_5\,{}^{64}Zn$: M^+, 783.3338.

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25. IR spectra of **1** and **2** at their film states which had the same visible spectra as in their aggregated solution showed that the bond of $Zn\cdots O3^1\text{-}H\cdots O{=}C13^1$ was present in the self-aggregated solid films.
26. Compound **1** has 15 fluorine atoms in a molecule, and the content of fluorine (25% (wt/wt)) is not so high that monomeric **1** could not be dissolved in pure HCFC225cb.
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