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Aggregation of Synthetic Zinc Chlorins with Several Esterified Alkyl Chains as Models of Bacteriochlorophyll-*c* Homologs

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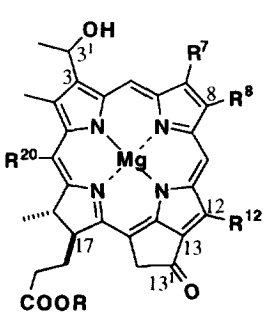
Abstract: Zinc complexes of 3-hydroxymethyl-13^1-oxochlorin possessing several branched alkyl chains as an esterified group at the 17-position were systematically prepared. In non-polar organic solvents, these compounds aggregated to form oligomers absorbing up to 800-nm light with around a 740-nm peak, which are good models for bacteriochlorophylls-*c* and *d*, extramembranous antenna pigments of photosynthetic green bacteria. All the visible spectra of the *in-vitro* oligomers are the same and the esterified alcohols induced no effect on the local structure of the oligomers. The circular dichroism spectra changed with elongation of esterified alkyl chains, which resulted in the similar spectra to the *in-vivo* oligomers. Esterified alcohols should subtly affect the supramolecular structure and/or stability of the self-aggregates. Copyright © 1996 Elsevier Science Ltd

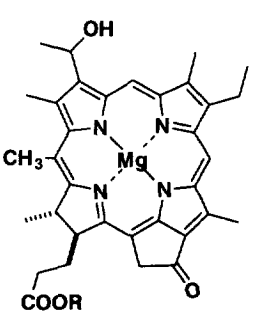
INTRODUCTION

Bacteriochlorophyll(=BChl)s-*c*, *d* and *e* are the main pigments for extramembranous light-harvesting antenna complexes (chlorosomes) of photosynthetic green bacteria[1]. These compounds self-aggregate in chlorosomes to form oligomers absorbing longer wavelength lights than the monomeric species[2,3]. Such *in-vivo* self-aggregates are structurally different from well-known pigment-peptide complexes in membranous antennae[4]. These self-aggregation arrangements are caused by the molecular structure of BChls-*c*, *d*, *e*, which have a 3^1-hydroxy group in the molecule. Bacteriochlorophylls-*a*, *b* and chlorophyll(=Chl)s-*a*, *b*, which are other photosynthetic antenna pigments, have less coordinating acetyl and vinyl groups at the 3-position,



BChl-*c*: R^7=R^{20}=CH_3
BChl-*d*: R^7=CH_3, R^{20}=H
BChl-*e*: R^7=CHO, R^{20}=CH_3

3^1-configuration=*R*, *S*
R^8=C_2H_5, $CH_2CH_2CH_3$,
$CH_2CH(CH_3)_2$, $CHC(CH_3)_3$
R^{12}=CH_3, C_2H_5
R=a long chain



BChl-*c* in *Chloroflexus aurantiacus*

Fig. 1. Chlorosomal pigments

respectively. Recent research[3,5-9] revealed that the special bonding 13-C=O···H–O···Mg is important for the self-aggregation.

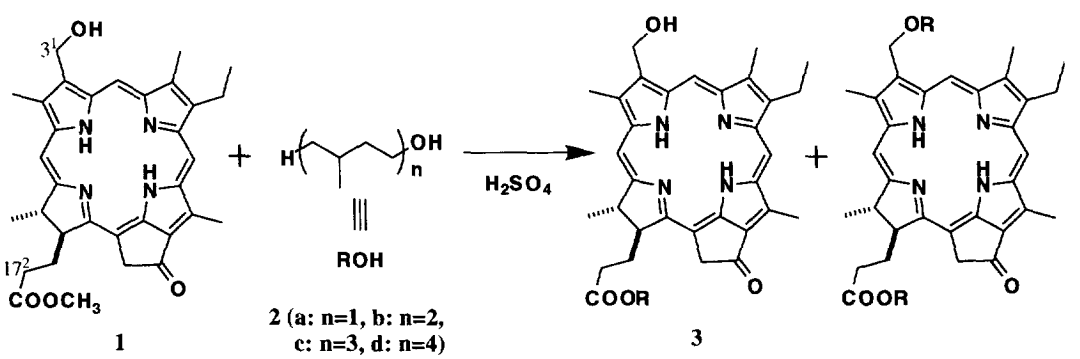
BChls-*c*, *d*, *e* are composed of several variants of molecular formula[10] as shown in Fig. 1, which are also different from the single molecular structures of (B)Chls-*a*, *b*. This divergency should regulate the *in-vivo* supramolecular structures and affect energy migration. Absolute configuration at the chiral 3^1-position[7,11-13] and alkyl groups at the 8- and 12-positions[11,14-16] control the *in-vitro* self-aggregation structures as well as the *in-vivo*. Moreover, some BChls-*c*, *d*, *e* have an only farnesyl group as an esterified alkyl chain at the 17-position and others have several alkyl chains[10,17,18]. Typically, in *Chloroflexus aurantiacus*, BChl-*c* has only ethyl and methyl groups at the 8- and 12-positions, respectively but several esterified chains, cetyl, oleyl, stearyl, geranylgeranyl and phytyl groups as a R-group in Fig. 1[17]. The natural diversity of esterified alcohol should have some biological meaning, especially for chlorosomal self-aggregates, but few attempts[19,20] to elucidate the reasons of the variety have been done to our knowledge.

We have already reported that zinc chlorins possessing hydroxymethyl group at the 3-position are good models for BChls-*c*, *d*, magnesium chlorins possessing 1-hydroxyethyl group at the 3-position[9,21,22]. The synthetic zinc chlorins are more available and stable than natural magnesium chlorins. Here we report on the systematic synthesis of zinc chlorins **7** with several esterified alkyl chains at the 17-position and the self-aggregation in non-polar organic solvents.

RESULTS AND DISCUSSION

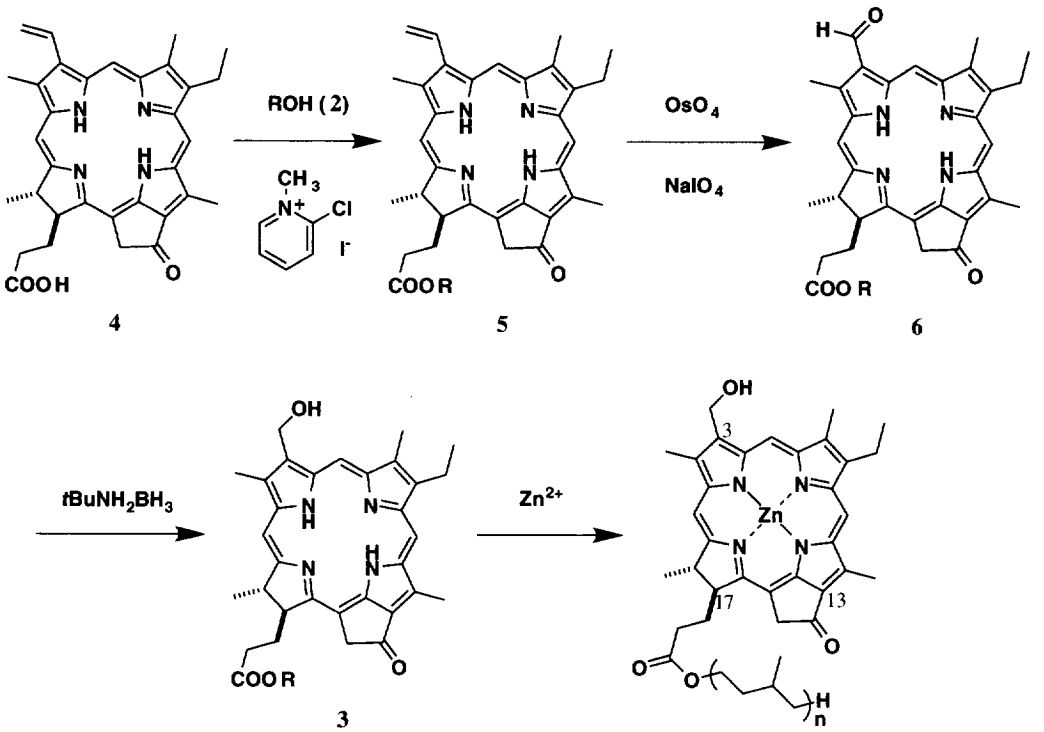
Synthesis

Methyl ester of **1** was exchanged to other esters **3** by treatment of ROH **2** and H_2SO_4 (see Scheme 1). The reaction was applied for C_5 and C_{10} alcohols (**2a** and **2b**) but neither C_{15} nor C_{20} ester (**3c** and **3d**) could be obtained by this route. The ester exchange afforded the desired product **3** with a small amount of an ether product as shown in Scheme 1. An alternative route is necessary for the effective preparation of **3**. Esterification of acid of **1** (17^2-COOH) with alcohol **2** without protection of the 3^1-hydroxy group was difficult and the synthetic route was chosen as shown in Scheme 2. Condensation of pyropheophorbide-*a* (**4**) with alcohol (C_5-C_{20}, **2a-d**) by 1-methyl-2-chloropyridinium iodide[23] gave the corresponding esters **5a-d** in high yield. The



1 **2 (a: n=1, b: n=2, c: n=3, d: n=4)** **3**

Scheme 1



Scheme 2

vinyl group of the esters **5** was oxidized by OsO_4-$NaIO_4$ to afford the aldehyde **6** and the formyl group was selectively reduced by tBuNH$_2$BH$_3$ to afford the hydroxymethyl group as in **3**[9]. Zinc metallation[9] of the compounds **3** gave the titled model compounds **7** possessing a branched alkyl group at the C17 ester group.

Visible Spectra

Zinc complexes **7a-d** in THF are monomeric species from the sharp visible absorption spectra[9] in which Q_y- and Soret bands are 646 and 424 nm, respectively (see a broken line of Fig. 2). In 1% (*v/v*) THF–hexane, all the four zinc complexes **7** showed the same visible spectra; λ_{max} (Q_y) = 734 and λ_{max} (Soret) = 449 nm (see a solid line of Fig. 2). The red-shifted and broadened spectra are characteristic of oligomeric **7** compared with other reported spectra[9]. The identification shows that **7a-d** aggregate in non-polar organic solvents to form an oligomer in the same manner, including 13-C=O···H(X)O···Zn and π-π interaction of chlorin chromophores which were reported so far[9]. Alkyl chains at the C17 ester do not affect the visible spectra of the *in-vitro* oligomers[24]. Larsen *et al.*[19] have already reported that visible spectra of *in-vivo* oligomers are independent upon the esterified alcohol (C_{10}-decanol and C_{20}-phytol) of BChl-*c* in a chlorosome. Theoretical study[25] suggested that 20 BChl-*c* molecules aggregate to reach the maximum value of red-shift in absorption spectra b exciton coupling and that aggregates of > 20 BChl-*c* show the similar visible spectra. Therefore alkyl groups the ester do not make a large contribution to the formation of self-aggregation[26] and also intermolecular interaction among the alkyl groups is not important for the visible absorption of aggregates (> 20 molecules).

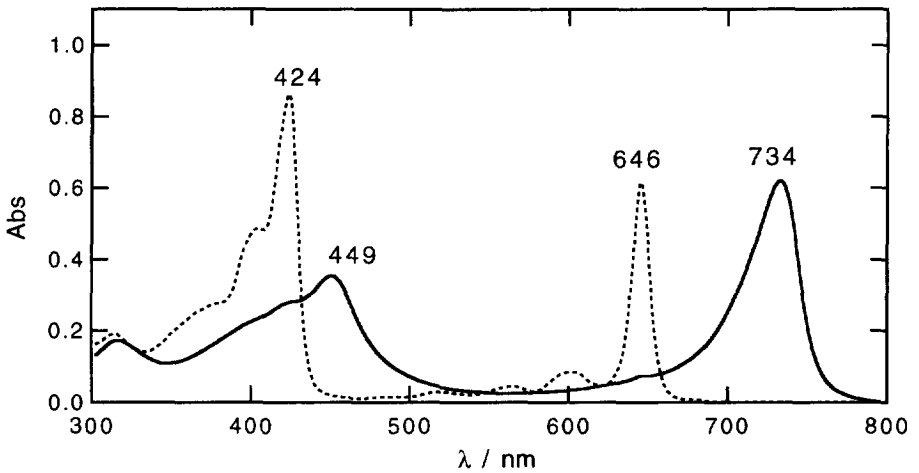


Fig. 2. UV-Vis spectra of **7**, —, in 1% (*v*/*v*) THF–hexane; ---, in THF.

Circular Dichroism Spectra

In 1% (*v*/*v*) THF–hexane of **7**, CD peaks were measured in the red-shifted absorption region. Especially, giant CD bands were observed in the red-shifted Q_y-band (see Fig. 3). The CD shapes are dependent upon the alkyl chain at the C17 ester. Oligomeric **7a** displayed the S-shape CD bands (type II spectra according to the nomenclature of Griebenow *et al.*[27]) as shown in Fig. 3B. The CD spectrum of oligomeric **7b** (a solid line of Fig. 3C) is different from that of **7a**. In **7b**, 745-nm minimum appeared and both the maximum peak around 730 nm and the minimum peak around 700 nm are small and/or blue-shifted compared with that of **7a**. These tendencies were observed in elongation of the alkyl chain, **7b**→**7c**→**7d**. Oligomeric **7c** and **7d** showed the reversed S-shape CD with a small dip at the lower wavelength region (mixture of major type I and minor type II) as shown in Fig. 3D and 3E. CD spectra of **7b** and **7c** (solid lines of Fig. 3C and 3D) were simulated by linear combination of those of **7a** with **7d** (broken lines of Fig. 3C and 3D), indicating that all the CD spectra observed were represented by combination of basic two components[27] (reversed S-shape type I spectra with the zero-crossing point ≈ absorption maximum and S-shape type II spectra with the zero-crossing point < absorption maximum). Systematic change in the CD spectra showed that supramolecular structures varied gradually with elongation of the esterified alkyl chain. Aggregates of mixture of BChls-*c* with C_{16}-C_{20} alkyl chains in natural chlorosomes of *Chloroflexus aurantiacus* J-10-fl[9] show a similar CD spectrum with *in-vitro* **7c** (with C_{15}) and **7d** (with C_{20}) oligomers. Therefore alkyl groups at the ester affect supramolecular structures and/or stability of self-aggregates. The effect might be ascribed to intra- and inter-supramolecular interaction of the alkyl groups in the self-aggregates or to the interaction of the alkyl chain with hexane as the solvent. Induced CD spectra by exciton coupling in the oligomers are still affected by aggregates of > 20 molecules, compared with the visible spectra. Considering that CD spectra are more sensitive to the supramolecular structure than visible spectra, the results of the change in CD spectra are consistent with no change in the visible spectra discussed above. Theoretical study also supports such a CD sensitivity[28].

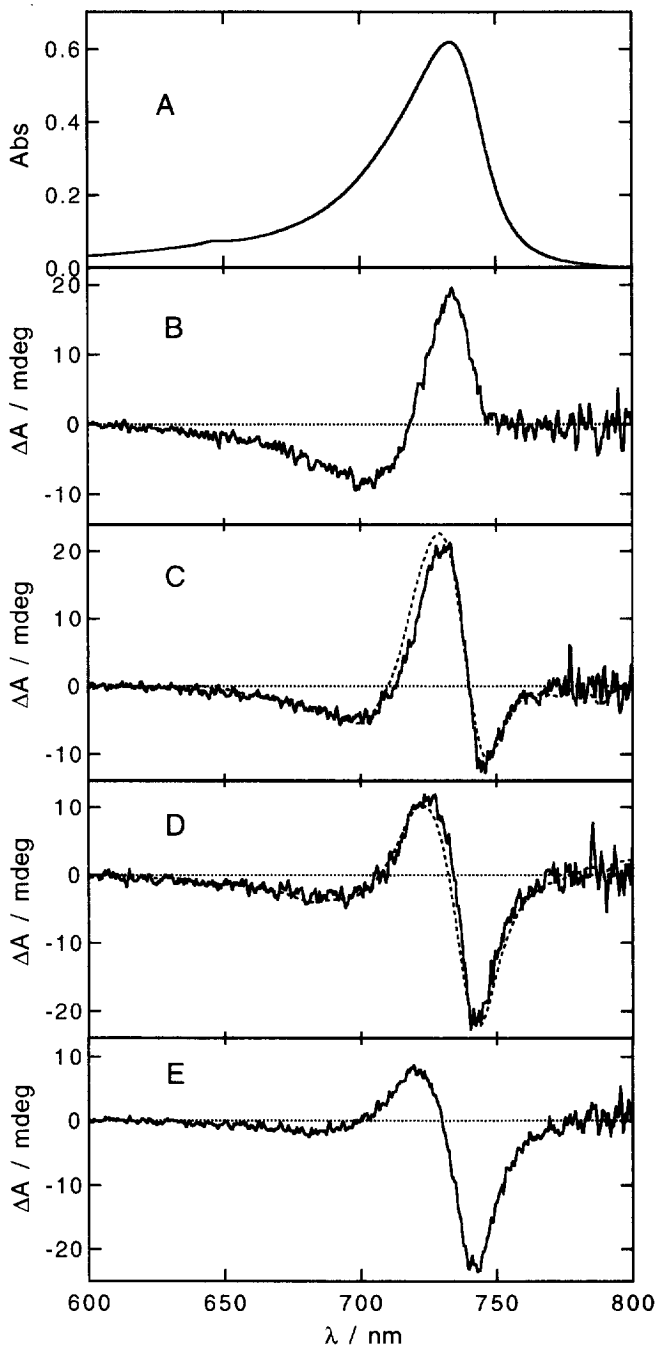


Fig. 3. Visible (A) and CD (B–E) spectra of **7** in 1% (*v*/*v*) THF–hexane,

A: VIS of **7**,

B: CD of **7a** (n=1, C_5),

C: —, CD of **7b** (n=2, C_{10}); ---, calculated by 1.21 (B) + 0.85 (E),

D: —, CD of **7c** (n=3, C_{15}); ---, calculated by 0.36 (B) + 1.15 (E),

E: CD of **7d** (n=4, C_{20}).

All CD spectra observed were normalized by absorbance of Q_y-peak, $\Delta A = (A_L - A_R) / Abs_{max}$.

CONCLUSION

Visible spectra showed that the supramolecular skeleton of self-aggregates of the synthetic zinc chlorins is unchanged even if the esterified alkyl chains are changed. CD spectra showed that the whole supramolecular structure is changed with the increase in the number of carbons in the alkyl chain at the C17 ester. Alkyl chains should affect aggregation number of **7** and large aggregates can make a stable helical structure to form a rod structure[29] which is seen in natural chlorosomes[1-3,15]. Similarity of CD spectra of *in-vitro* oligomer of **7c** and **7d** with *in-vivo* oligomers of BChl-*c* shows that long alkyl chains stabilize such a rod structure. The present model study clarifies that the long alkyl chains ($\geq C_{15}$) are important for chlorosome-like self-aggregation and indicates that the *in-vivo* diversity of esterified alcohols should regulate the supramolecular structures, including rod structures.

EXPERIMENTAL

Apparatus

Visible absorption spectra were measured with Hitachi U-3500 spectrophotometer. Circular dichroism spectra were measured with Jasco J-720W spectropolarimeter. All melting points were measured with a Yanagimoto micro melting apparatus and were uncorrected. ^{1}H-NMR spectra were measured with Bruker AC-300 spectrometer; chemical shifts (δ) are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) as an internal reference. Mass spectra were recorded on JEOL HX-100 spectrometer; FAB-MS samples were dissolved in $CHCl_3$ and *m*-nitrobenzyl alcohol was used as the matrix. High-performance liquid chromatography was done with Shimadzu LC-10AS pump, SPD-10AV visible detector and C-R6A chromatopac.

Materials

Methyl 3-devinyl-3-hydroxymethylpyropheophorbide-*a* (**1**) and pyropheophorbide-*a* (**4**) were prepared according to the procedures reported by Tamiaki *et al.*[9] 3-Methyl-1-butanol (**2a**) and 3,7-dimethyl-1-octanol (**2b**) were commercially available. 3,7,11-Trimethyl-1-dodecanol (**2c**) and 3,7,11,15-tetramethyl-1-hexadecanol (**2d**) were prepared by hydrogenation of farnesol and phytol, respectively (stirring of the methanol solution with palladium-charcoal overnight under H_2 at room temperature). Toluene was distilled and stored over molecular sieves 3A. THF was distilled from CaH_2 before use. Flash column chromatography was performed with silica gel (Merck, Kieselgel 60, 9385). High-performance liquid chromatography was performed with a packed ODS column (Gelpack, GL-OP100, Hitachi Chemical Co., 6 × 150 mm). Solvents for visible and CD spectra were purchased from Nacalai Tesque (Grade for UV-Spectroscopy).

General Procedures

Ester Exchange of Methyl to a Long Chain Group. Methyl 3-devinyl-3-hydroxymethylpyropheophorbide-*a* (**1**, 20 mg) was added to an alcohol (**2a** and **2b**, 8 ml). Concentrated H_2SO_4 (0.8 ml) was dropwise added to the reaction mixture at 0 °C. After stirred overnight under Ar, the mixture was poured into ice water and dichloromethane, and stirred. The aqueous layer was extracted with dichloromethane repeatedly

until it was colorless. The combined organic layers were washed with aq. $NaHCO_3$ and water, and dried over Na_2SO_4. Dichloromethane and the unreacted alcohol was evaporated *in vacuo*. The residue was chromatographed with Et_2O–CH_2Cl_2, to give the corresponding ester of 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (**3a** and **3b**) with a small amount of an ether product as shown in Scheme 1.

Condensation of Pyropheophorbide-*a* with a Saturated Alcohol. Distilled toluene (20 ml) and triethylamine (260 μl, 10 eq.) and 1-methyl-2-chloropyridinium iodide (240 mg, 5 eq.) were added to a THF (20 ml) solution of pyropheophorbide-*a* (**4**, 100 mg). To the reaction mixture was added an equivalent of a saturated alcohol **2** with stirring. The reaction mixture was refluxed for 10 h under Ar. After cooling, the mixture was poured into water and dichloromethane, and stirred. The aqueous layer was extracted with dichloromethane repeatedly until it was colorless. The combined organic layers were washed with an aqueous saturated $NaHCO_3$ solution and water, and dried over Na_2SO_4. After evaporation, the residue was chromatographed with 4% Et_2O–CH_2Cl_2, to give the saturated alcohol ester of pyropheophorbide-*a* (**5**).

Oxidation of 3-Vinyl to 3-Formyl Group. An aqueous (7.5 ml) solution of $NaIO_4$ (1.19 g) and acetic acid (0.5 ml) was added with a peristaltic pump to a distilled THF solution (100 ml) of a compound possessing 3-vinyl group (**5**, 1 mmol) and OsO_4 (10 mg). After stirred overnight under Ar, the reaction mixture was poured into water and CH_2Cl_2 and was stirred for 30 min. The aqueous layer was extracted with CH_2Cl_2 repeatedly until it was colorless. The combined organic layers were washed with an aqueous saturated $NaHCO_3$ solution and water, and dried over Na_2SO_4. After evaporation, the residue was chromatographed with 2% Et_2O–CH_2Cl_2 to give the compound possessing 3-formyl group (**6**).

Reduction of 3-Formyl to 3-Hydroxymethyl Group. *tert*-Butylamine borane complex (20 mg) was added to a dichloromethane (100 ml) solution of a compound possessing 3-formyl group (**6**, 0.2 mmol). After stirred overnight under Ar, the mixture was poured into aq. 2% HCl (100 ml) and stirred for 20 min in an ice bath. The aqueous layer was extracted with dichloromethane repeatedly until it was colorless. The combined organic layers were washed with aq. 2% HCl (500 ml), water (500 ml), aq. $NaHCO_3$ (100 ml) and aq. NaCl (500 ml), and dried over Na_2SO_4. After evaporation, the residue was chromatographed with 8-10% Et_2O–CH_2Cl_2, to give the compound possessing 3-hydroxymethyl group (**3**).

Zinc Metallation of a Chlorin. A saturated methanol solution with $Zn(OAc)_2{\cdot}2H_2O$ (1 ml) was added to a dichloromethane (10 ml) solution of metal-free chlorin (**3**, 10 mg). After stirred overnight, the reaction mixture was poured into aq. 4% $NaHCO_3$, and stirred for 15 min. After filtration, the mixture was extracted with dichloromethane, washed with water and dried over Na_2SO_4. After evaporation, the residue was chromatographed with 2% MeOH–CH_2Cl_2, to give the corresponding zinc complex (**7**). The zinc complexes were highly purified by HPLC (methanol as an eluate) before spectroscopic analysis.

Spectral Data

Pyropheophorbide-*a* 3-methyl-1-butyl ester (5a). 69% yield (based on **4**); black solids; mp 85-90 °C; UV-Vis (CH_2Cl_2) λ_{max}=667 (relative intensity, 0.43), 610 (0.07), 539 (0.09), 509 (0.10), 413 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.51, 9.39, 8.56 (1H+1H+1H, s, 5-, 10-, 20-H), 8.01 (1H, dd, *J*=12, 18 Hz, 3-

CH), 6.29 (1H, dd, *J*=1, 18 Hz, 3^1-CH-trans), 6.18 (1H, dd, *J*=1, 12 Hz, 3^1-CH-cis), 5.27, 5.11 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.49 (1H, dq, *J*=2, 7 Hz, 18-H), 4.31 (1H, dt, *J*=8, 2 Hz, 17-H), 3.99, 4.05 (1H+1H, dt, *J*=11, 7 Hz, 17^4-CH_2), 3.69 (2H, q, *J*=7 Hz, 8-CH_2), 3.68, 3.41, 3.24 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.60-2.75, 2.43-2.58, 2.17-2.38 (1H+1H+2H, m, 17-CH_2CH_2), 1.82 (3H, d, *J*=7 Hz, 18-CH_3), 1.70 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.46-1.65 (1H, m, 17^6-CH), 1.38 (2H, q, *J*=7 Hz, 17^5-CH_2), 0.82 (6H, d, *J*=7 Hz, 17^7-$(CH_3)_2$), 0.46 (1H, br, NH), -1.69 (1H, s, NH). MS (FAB) found: *m/z* 605. Calcd for $C_{38}H_{45}N_4O_3$: MH^+, 605.

Pyropheophorbide-*a* 3,7-dimethyl-1-octyl ester (5b). 76% yield (based on **4**); black solids; mp 67-72 °C; UV-Vis (CH_2Cl_2) λ_{max}=667 (0.44), 610 (0.07), 539 (0.09), 509 (0.10), 414 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.50, 9.39, 8.56 (1H+1H+1H, s, 5-, 10-, 20-H), 8.01 (1H, dd *J*=12, 18 Hz, 3-CH), 6.29 (1H, dd, *J*=1, 18 Hz, 3^1-CH-trans), 6.17 (1H, dd, *J*=1, 12 Hz, 3^1-CH-cis), 5.28, 5.11 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.49 (1H, dq, *J*=2, 7 Hz, 18-H), 4.31 (1H, dt, *J*=8, 2 Hz, 17-H), 3.99, 4.05 (1H+1H, dt, *J*=11, 7 Hz, 17^4-CH_2), 3.69 (2H, q, *J*=7.5 Hz, 8-CH_2), 3.67, 3.41, 3.24 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.65-2.79, 2.48-2.65, 2.20-2.45 (1H+1H+2H, m, 17-CH_2CH_2), 1.82 (3H, d, *J*=7 Hz, 18-CH_3), 1.70 (3H, t, *J*=7.5 Hz, 8^1-CH_3), 0.95-1.55, (10H, m, $17^{5,7-9}$-CH_2, $17^{6,10}$-CH), 0.79 (9H, d, *J*=7 Hz, 17^7-CH_3, 17^{11}-$(CH_3)_2$), 0.49 (1H, br, NH), -1.68 (1H, s, NH). MS (FAB) found: *m/z* 675. Calcd for $C_{43}H_{55}N_4O_3$: MH^+, 675.

Pyropheophorbide-*a* 3,7,11-trimethyl-1-dodecyl ester (5c). 50% yield (based on **4**); black solids; mp 61-65 °C; UV-Vis (CH_2Cl_2) λ_{max}=668 (0.47), 610 (0.08), 539 (0.09), 509 (0.10), 414 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.50, 9.39, 8.56 (1H+1H+1H, s, 5-, 10-, 20-H), 8.01 (1H, dd *J*=12, 18 Hz, 3-CH), 6.28 (1H, dd, *J*=1, 17 Hz, 3^1-CH-trans), 6.17 (1H, dd, *J*=1, 12 Hz, 3^1-CH-cis), 5.28, 5.11 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.49 (1H, dq, *J*=2, 7 Hz, 18-H), 4.31 (1H, dt, *J*=8, 2 Hz, 17-H), 3.99, 4.06 (1H+1H, m, 17^4-CH_2), 3.69 (2H, q, *J*=8 Hz, 8-CH_2), 3.67, 3.40, 3.23 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.65-2.73, 2.47-2.57, 2.20-2.45 (1H+1H+2H, m, 17-CH_2CH_2), 1.82 (3H, d, *J*=7 Hz, 18-CH_3), 1.70 (3H, t, *J*=8 Hz, 8^1-CH_3), 0.93-1.58 (17H, m, $17^{5,7-9,11-13}$-CH_2, $17^{6,10,14}$-CH), 0.86, 0.76, 0.79 (6H+3H+3H, d, *J*=7 Hz, $17^{7,11}$-CH_3, 17^{15}-$(CH_3)_2$), 0.48 (1H, br, NH), -1.68 (1H, s, NH). MS (FAB) found: *m/z* 745. Calcd for $C_{48}H_{65}N_4O_3$: MH^+, 745.

Pyropheophorbide-*a* 3,7,11,15-tetramethyl-1-hexadecyl ester (5d). 45% yield (based on **4**); black solids; mp 58-61 °C; UV-Vis (CH_2Cl_2) λ_{max}=667 (0.43), 610 (0.07), 539 (0.08), 509 (0.09), 414 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.52, 9.41, 8.57 (1H+1H+1H, s, 5-, 10-, 20-H), 8.02 (1H, dd *J*=12, 18 Hz, 3-CH), 6.29 (1H, dd, *J*=1, 18 Hz, 3^1-CH-trans), 6.18 (1H, dd, *J*=1, 12 Hz, 3^1-CH-cis), 5.28, 5.11 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.50 (1H, dq, *J*=2, 7 Hz, 18-H), 4.31 (1H, dt *J*=8, 2 Hz, 17-H), 3.99, 4.05 (1H+1H, dt, *J*=11, 7 Hz, 17^4-CH_2), 3.70 (2H, q, *J*=8 Hz, 8-CH_2), 3.68, 3.41, 3.25 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.62-2.77, 2.44-2.60, 2.20-2.38 (1H+1H+2H, m, 17-CH_2CH_2), 1.82 (3H, d, *J*=7 Hz, 18-CH_3), 1.70 (3H, t, *J*=8 Hz, 8^1-CH_3), 0.90-1.62 (24H, m, $17^{5,7-9,11-13,15-17}$-CH_2, $17^{6,10,14,18}$-CH), 0.86, 0.84, 0.82, 0.80 (3H+6H+3H+3H, d, *J*=7 Hz, $17^{7,11,15}$-CH_3, 17^{19}-$(CH_3)_2$), 0.49 (1H, br, NH), -2.29 (1H, s, NH). MS (FAB) found: *m/z* 815. Calcd for $C_{53}H_{75}N_4O_3$: MH^+, 815.

Pyropheophorbide-*d* 3-methyl-1-butyl ester (6a). 60% yield (based on **5a**); dark brown solids; mp 95-100 °C; UV-Vis (CH_2Cl_2) λ_{max}=695 (0.79), 555 (0.15), 522 (0.13), 428 (1.00), 388 nm (0.85); ^{1}H-NMR ($CDCl_3$) δ=11.42 (1H, s, 3-CH), 10.06, 9.38, 8.79 (1H+1H+1H, s, 5-, 10-, 20-H), 5.32, 5.17 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.57 (1H, dq, *J*=2, 7 Hz, 18-H), 4.31 (1H, dt, *J*=8, 2 Hz, 17-H), 4.01, 4.08 (1H+1H, dt, *J*=11, 7 Hz, 17^4-CH_2), 3.71, 3.62 3.16 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 3.56 (2H, q, *J*=7 Hz, 8-CH_2), 2.65-2.78, 2.52-2.65, 2.23-2.41 (1H+1H+2H, m, 17-CH_2CH_2), 1.87 (3H, d, *J*=7 Hz, 18-CH_3), 1.60 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.45-1.6 (1H, m, 17^6-CH), 1.4 (2H, q, *J*=7 Hz, 17^5-CH_2), 0.84 (6H, d, *J*=7 Hz, 17^7-$(CH_3)_2$), -0.35 (1H, br, NH), -1.69 (1H, s, NH). MS(FAB) found: *m/z* 607. Calcd for $C_{37}H_{43}N_4O_4$: MH^+, 607.

Pyropheophorbide-*d* 3,7-dimethyl-1-octyl ester (6b). 59% yield (based on **5b**); dark brown solids; mp 85-88 °C; UV-Vis (CH_2Cl_2) λ_{max}=695 (0.90), 555 (0.14), 522 (0.14), 428 (1.00), 388 nm (0.86); ^{1}H-NMR ($CDCl_3$) δ=11.40 (1H, s, 3-CH), 10.08, 9.41, 8.79 (1H+1H+1H, s, 5-, 10-, 20-H), 5.34, 5.17 (2H, d, *J*=20 Hz, 13^1-CH_2), 4.57 (1H, dq, *J*=2, 7 Hz, 18-H), 4.31 (1H, dt, *J*=8, 2 Hz, 17-H), 4.00, 4.08 (1H+1H, dt, *J*=11, 7 Hz, 17^4-CH_2), 3.72, 3.63 3.17 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 3.58 (2H, q, *J*=7 Hz, 8-CH_2), 2.65-2.83, 2.52-2.65, 2.21-2.41 (1H+1H+2H, m, 17-CH_2CH_2), 1.87 (3H, d, *J*=7 Hz, 18-CH_3), 1.64 (3H, t, *J*=8 Hz, 8^1-CH_3), 0.95-1.58 (10H, m, $17^{5,7\text{-}9}$-CH_2, $17^{6,10}$-CH), 0.80 (9H, d, *J*=7 Hz, 17^7-CH_3, 17^{11}-$(CH_3)_2$), -0.33 (1H, br, NH), -2.27 (1H, s, NH). MS (FAB) found: *m/z* 677. Calcd for $C_{42}H_{53}N_4O_4$: MH^+, 677.

Pyropheophorbide-*d* 3,7,11-trimethyl-1-dodecyl ester (6c). 58% yield (based on **5c**); dark brown solids; mp 68-72 °C; UV-Vis (CH_2Cl_2) λ_{max}=694 (0.74), 554 (0.15), 522 (0.15), 427 (1.00), 388 nm (0.89); ^{1}H-NMR ($CDCl_3$) δ=11.40 (1H, s, 3-CH), 10.04, 9.37, 8.79 (1H+1H+1H, s, 5-, 10-, 20-H), 5.33, 5.17 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.58 (1H, dq, *J*=2, 7 Hz, 18-H), 4.39 (1H, dt, *J*=8, 2 Hz, 17-H), 4.02, 4.08 (1H+1H, dt, *J*=11, 7 Hz, 17^4-CH_2), 3.71, 3.62, 3.15 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 3.58 (2H, q, *J*=7 Hz, 8-CH_2), 2.70-2.81, 2.51-2.68, 2.23-2.40 (1H+1H+2H, m, 17-CH_2CH_2), 1.88 (3H, d, *J*=7 Hz, 18-CH_3), 1.63 (3H, t, *J*=8 Hz, 8^1-CH_3), 0.96-1.68 (17H, m, $17^{5,7\text{-}9,11\text{-}13}$-$CH_2$, $17^{6,10,14}$-CH), 0.83, 0.82, 0.78 (6H+3H+3H, d, *J*=6.5 Hz, $17^{7,11}$-CH_3, 17^{15}-$(CH_3)_2$), -0.38 (1H, br, NH), -2.30 (1H, s, NH). MS(FAB) found: *m/z* 747. Calcd for $C_{47}H_{63}N_4O_4$: MH^+, 747.

Pyropheophorbide-*d* 3,7,11,15-tetramethyl-1-hexadecyl ester (6d). 61% yield (based on **5d**); dark brown solids; mp 62-66 °C; UV-Vis (CH_2Cl_2) λ_{max}=695 (0.78), 554 (0.17), 522 (0.17), 428 (1.00), 388 nm (0.94); ^{1}H-NMR ($CDCl_3$) δ=11.52 (1H, s, 3-CH), 10.25, 9.55, 8.83 (1H+1H+1H, s, 5-, 10-, 20-H), 5.35, 5.19 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.58 (1H, dq, *J*=2, 7 Hz, 18-H), 4.39 (1H, dt *J*= 2, 8 Hz, 17-H), 3.95-4.09 (1H+1H, m, 17^4-CH_2), 3.76, 3.70, 3.28 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 3.69 (2H, q, *J*=8 Hz, 8-CH_2), 2.66-2.80, 2.51-2.65, 2.23-2.38 (1H+1H+2H, m, 17-CH_2CH_2), 1.86 (3H, d, *J*=7 Hz, 18-CH_3), 1.70 (3H, t, *J*=8 Hz, 8^1-CH_3), 0.94-1.68 (24H, m, $17^{5,7\text{-}9,11\text{-}13,15\text{-}17}$-$CH_2$, $17^{6,10,14,18}$-CH), 0.87, 0.84, 0.80, 0.79 (3H+6H+3H+3H, d, *J*=7 Hz, $17^{7,11,15}$-CH_3, 17^{19}-$(CH_3)_2$), -0.2 (1H, br, NH), -2.10 (1H, s, NH). MS(FAB) found: *m/z* 817. Calcd for $C_{52}H_{73}N_4O_4$: MH^+, 817.

3-Devinyl-3-hydroxymethylpyropheophorbide-*a* 3-methyl-1-butyl ester (3a). 81% yield (based on **6a**); bluish black solids; mp 120-123 °C; UV-Vis (CH_2Cl_2) λ_{max}=662 (0.43), 605 (0.06), 536 (0.08), 504 (0.08), 409 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.49, 9.44, 8.56 (1H+1H+1H, s, 5-, 10-, 20-H), 5.91 (2H, s, 3-CH_2), 5.25, 5.09 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.48 (1H, dq, *J*=2, 7 Hz, 18-H), 4.28 (1H, dt, *J*=8, 2 Hz, 17-H), 4.00, 4.06 (1H+1H, dt, *J*=11, 7 Hz, 17^4-CH_2), 3.69 (2H, q, *J*=8 Hz, 8-CH_2), 3.65, 3.29 3.26 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.59-2.75, 2.44-2.59, 2.18-2.36 (1H+1H+2H, m, 17-CH_2CH_2), 1.80 (3H, d, *J*=7 Hz, 18-CH_3), 1.69 (3H, t, *J*=8 Hz, 8^1-CH_3), 1.45-1.61 (1H, m, 17^6-CH), 1.38 (2H, q, *J*=7 Hz, 17^5-CH_2), 0.83 (6H, d, *J*=7 Hz, 17^7-$(CH_3)_2$), 0.0 (1H, br, NH), -1.69 (1H, s, NH). MS (FAB) found: *m/z* 608. Calcd for $C_{37}H_{45}N_4O_4$: MH^+, 608.

3-Devinyl-3-hydroxymethylpyropheophorbide-*a* 3,7-dimethyl-1-octyl ester (3b). 83% yield (based on **6b**); bluish black solids; mp 115-118 °C; UV-Vis (CH_2Cl_2) λ_{max}=662 (0.47), 605 (0.07), 536 (0.09), 505 (0.09), 410 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.44, 9.42, 8.55 (1H+1H+1H, s, 5-, 10-, 20-H), 5.88 (2H, s, 3-CH_2), 5.22, 5.07 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.47 (1H, dq, *J*=2, 7 Hz, 18-H), 4.26 (1H, br-d, *J*=8 Hz, 17-H), 3.97-4.09 (2H, m, 17^4-CH_2), 3.68 (2H, q, *J*= 7.5 Hz, 8-CH_2), 3.62, 3.41 3.25 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.60-2.75, 2.48-2.60, 2.18-2.35 (1H+1H+2H, m, 17-CH_2CH_2), 1.79 (3H, d, *J*=7 Hz, 18-CH_3), 1.68 (3H, t, *J*=7.5 Hz, 8^1-CH_3), 1.07-1.62 (10H, m, 17$^{5,7\text{-}9}$-CH_2, 17^{6,10}-CH), 0.89, 0.87 (6H+3H, d, *J*=7 Hz, 17^7-CH_3, 17^{11}-$(CH_3)_2$), 0.0 (1H, br, NH), -1.83 (1H, s, NH). MS (FAB) found: *m/z* 678. Calcd for $C_{42}H_{55}N_4O_4$: MH^+, 678.

3-Devinyl-3-hydroxymethylpyropheophorbide-*a* 3,7,11-trimethyl-1-dodecyl ester (3c). 80% yield (based on **6c**); bluish black solids; mp 113-115°C; UV-Vis (CH_2Cl_2) λ_{max}=662 (0.48), 605 (0.08), 536 (0.08), 505 (0.09), 410 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.32, 9.24, 8.50 (1H+1H+1H, s, 5-, 10-, 20-H), 5.78 (2H, s, 3-CH_2), 5.10, 4.96 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.41 (1H, dq, *J*=2, 7 Hz, 18-H), 4.06 (1H, br-d, *J*=8 Hz, 17-H), 4.01-4.12 (2H, m, 17^4-CH_2), 3.59 (2H, q, *J*=8 Hz, 8-CH_2), 3.49, 3.39, 3.19 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.55-2.65, 2.45-2.55, 2.10-2.32 (1H+1H+2H, m, 17-CH_2CH_2), 1.75 (3H, d, *J*=7 Hz, 18-CH_3), 1.63 (3H, t, *J*=8 Hz, 8^1-CH_3), 0.81-1.59 (17H, m, 17$^{5,7\text{-}9,11\text{-}13}$-$CH_2$, 17^{6,10,14}-CH), 0.83, 0.78 (9H+3H, d, *J*=6.5 Hz, 17^{7,11}-CH_3, 17^{15}-$(CH_3)_2$), 0.0 (1H, br, NH), -2.00 (1H, s, NH). MS (FAB) found: *m/z* 749. Calcd for $C_{47}H_{65}N_4O_4$: MH^+, 749.

3-Devinyl-3-hydroxymethylpyropheophorbide-*a* 3,7,11,15-tetramethyl-1-hexadecyl ester (3d). 81% yield (based on **6d**); bluish black solids; mp 112-115 °C; UV-Vis (CH_2Cl_2) λ_{max}=662 (0.46), 605 (0.07), 536 (0.08), 505 (0.09), 410 nm (1.00); ^{1}H-NMR ($CDCl_3$) δ=9.37, 9.34, 8.53 (1H+1H+1H, s, 5-, 10-, 20-H), 5.85 (2H, s, 3-CH_2), 5.17, 5.01 (1H+1H, d, *J*=20 Hz, 13^1-CH_2), 4.45 (1H, q, *J*=7 Hz, 18-H), 4.39 (1H, d, *J*=8 Hz, 17-H), 3.97-4.13 (2H, m, 17^4-CH_2), 3.64 (2H, q, *J*=8 Hz, 8-CH_2), 3.56, 3.40, 3.23 (3H+3H+3H, s, 2-, 7-, 12-CH_3), 2.48-2.58, 2.38-2.48, 2.07-2.22 (1H+1H+2H, m, 17-CH_2CH_2), 1.78 (3H, d, *J*=7 Hz, 18-CH_3), 1.66 (3H, t, *J*=8 Hz, 8^1-CH_3), 0.96-1.60 (24H, m, 17$^{5,7\text{-}9,11\text{-}13,15\text{-}17}$-$CH_2$, 17^{6,10,14,18}-CH), 0.88, 0.83, 0.80, 0.79 (6H+3H+3H+3H, d, *J*=7 Hz, 17^{7,11,15}-CH_3, 17^{19}-$(CH_3)_2$), 0.1 (1H, br, NH), -1.92 (1H, s, NH). MS (FAB) found: *m/z* 819. Calcd for $C_{52}H_{75}N_4O_4$: MH^+, 819.

Zinc 3-devinyl-3-hydroxymethylpyropheophorbide-*a* 3-methyl-1-butyl ester (7a). 92% yield (based on **3a**); dark green solids; mp > 300 °C; UV-Vis (THF) λ_{max} =647 (0.73), 602 (0.09), 566 (0.05), 521 (0.03), 424 nm (1.00). MS (FAB) found: *m/z* 670. Calcd for $C_{37}H_{42}N_4O_4{}^{64}Zn$: M^+, 670.

Zinc 3-devinyl-3-hydroxymethylpyropheophorbide-*a* 3,7-dimethyl-1-octyl ester (7b). 92% yield (based on **3b**); dark green solids; mp > 300 °C; UV-Vis (THF) λ_{max} =647 (0.71), 602 (0.09), 566 (0.05), 521 (0.03), 424 nm (1.00). MS (FAB) found: *m/z* 740. Calcd for $C_{42}H_{52}N_4O_4{}^{64}Zn$: M^+, 740.

Zinc 3-devinyl-3-hydroxymethylpyropheophorbide-*a* 3,7,11-trimethyl-1-dodecyl ester (7c). 94% yield (based on **3c**); dark green solids; mp > 300 °C; UV-Vis (THF) λ_{max} =647 (0.73), 602 (0.11), 566 (0.05), 521 (0.03), 424 nm (1.00). MS (FAB) found: *m/z* 810. Calcd for $C_{47}H_{62}N_4O_4{}^{64}Zn$: M^+, 810.

Zinc 3-devinyl-3-hydroxymethylpyropheophorbide-*a* 3,7,11,15-tetramethyl-1-hexadecyl ester (7d). 95% yield (based on **3d**); dark green solids; mp > 300 °C; UV-Vis (THF) λ_{max}=647 (0.73), 602 (0.09), 566 (0.05), 521 (0.03), 424 nm (1.00). MS (FAB) found: *m/z* 880. Calcd for $C_{52}H_{72}N_4O_4{}^{64}Zn$: M^+, 880.

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