Synthesis of a Novel Cyclic Chlorophyll Hetero-Dyad as a Model Compound for Stacked Chlorophylls Found in Photosynthetic Systems

Nobuo Kosaka[a] and Hitoshi Tamiaki*[a]

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A covalently doubly linked cyclic chlorophyll hetero-dyad was stereospecifically prepared as a model for naturally stacked chlorophyll suprastructures. In the synthetic dyad, two composite macrocycles are situated very close to each other such that the Q_y transition dipole moments intramolecularly interact to give intense circular dichroism peaks.

Introduction

Chlorophyll is present in primary photosynthetic systems, light-harvesting antennae, and reaction centers, and plays a principal role in a series of light-harvesting, energy transfer, and charge transfer processes. Its specific arrangement causes significant interactions among the chlorophyll pigments that are highly effective for these processes. Some X-ray crystallographic analyses have already revealed supramolecular structures of the primary photosynthetic apparatus at an atomic level.[1−3] In the crystal structures, many "stacked" chlorophylls are found, as in a special pair of bacterial reaction centers[1] and the B850 ring of light-harvesting 2 complexes.[2] Studying the special properties of naturally occurring stacked chlorophylls is meaningful for the exploration of the mechanisms of initial photosynthetic processes. While the importance of the stacked supramolecular structures of chlorophylls has been recognized, particular features such as the orientation-dependence of the chlorophyll pigments remains a matter of debate and ongoing investigation. Such unresolved issues inspired us to investigate them further.

In order to understand the characteristic features in the naturally stacked suprastructures composed of multiple chlorophylls, we have explored the physical properties of novel stacked chlorophyll dyads prepared systematically by simple procedures. Various covalently-linked chlorophyll dimers have been reported,[4−14] but only two reports, to the best of our knowledge, on the syntheses of "cyclic" chlorophyll dyads where the chlorin macrocycles are covalently and doubly linked to each other are available,[5,7] and no cyclic chlorophyll "hetero"-dyad has yet been reported. Doubly linked chlorophyll moieties provide a well-defined molecular structure, even in a broad range of solvents and temperatures, and therefore, the physical properties of such a conformationally frozen molecule can be explored by various techniques. This advantage is useful for the investigation of other special properties of the stacked structure of chlorophylls.

Systematic preparation of stacked chlorophyll hetero-dyads with a different stacking arrangements and orientations is thus important for the understanding of their properties. Here, we report on the synthesis and characterization of a novel cyclic chlorophyll hetero-dyad, in which the two chlorin macrocycles are doubly linked and close to each other. The present synthetic strategy is so simple such that it would provide a series of cyclic chlorophyll dyads.

Results and Discussion

The cyclic chlorophyll hetero-dyad **9** was prepared according to Scheme 1, as a model for the doubly linked cyclic chlorophyll dyads for the stacked chlorophylls in photosynthetic systems. Chlorophyll-*a* extracted from a cyanobacterium, *Spirulina geitleri*, was converted into methyl pheophorbide-*a* (13^2R-epimer of **1**), and a small amount of the 13^2S-epimer (*a*′) was also formed.[11] After removal of the 13^2-methoxycarbonyl group in **1**, oxidative cleavage of the 3-vinyl group in the resulting compound **2**[11,15] and successive acidic hydrolysis of methyl ester **3** afforded pyropheophorbide-*d* (**4**). Transformation of the 3-vinyl group in **1** to the 3-hydroxymethyl group in **6** (via the 3-formyl group in **5**) was achieved by procedures reported previously.[16] Analysis of the ^{1}H NMR spectrum of the resulting alcohol **6** shows a mixture of the 13^2R- (*a*) and *S*-epimers (*a*′) (ca. 6:1). Esterification of the 17-propionic acid side chain in **4** with the 3-hydroxymethyl group in **6** gave a singly-linked linear chlorophyll dyad **7** in a 89% yield, using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrogen chloride (EDC·HCl) as a coupling reagent and 4-(*N*,*N*-dimethylami-

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[a] Department of Bioscience and Biotechnology, Faculty of Science and Engineering,
Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan
Fax: (internat.) +81-77-561-2659
E-mail: tamiaki@se.ritsumei.ac.jp

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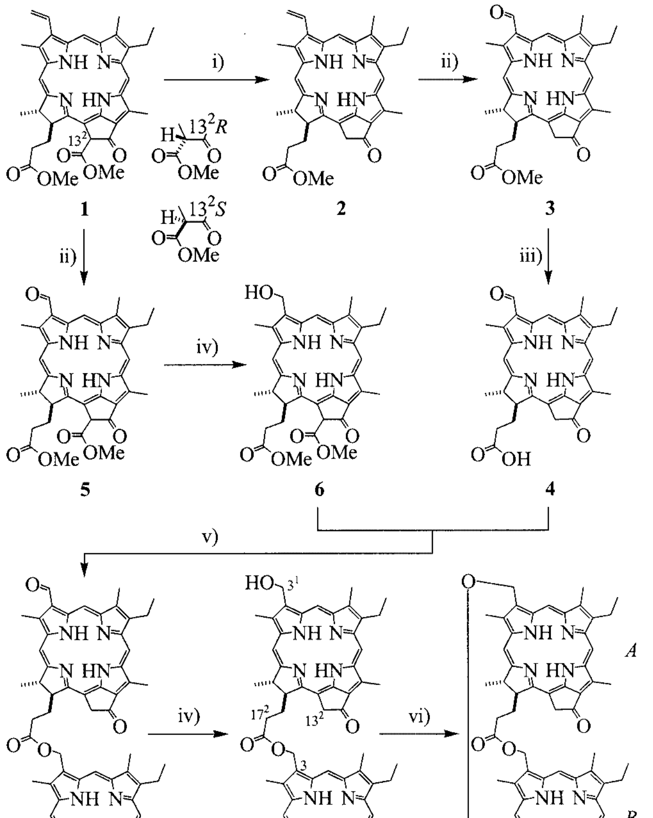
no)pyridine (DMAP) as a coupling promoter agent in dichloromethane.[14] The 3-formyl group in **7** reacted with *tert*-butylamine borane complex ($t\mathrm{BuNH_2BH_3}$) in dichloromethane to give the corresponding alcohol **8** in a 88% yield. Both synthetic singly-linked dyads **7** and **8** were ca. 6:1 $13^2R/S$ mixtures, as confirmed by their 13^2-H signals (see Exp. Sect.). Intramolecular β-keto transesterification of **8** was readily accomplished at low concentration (45 μM) by action of 2-chloro-1-methylpyridinium iodide (CMPI) and DMAP in refluxing toluene,[12,17,18] to give the doubly linked cyclic chlorophyll dyad **9** in a 37% yield (**8** was completely used up). Under the above conditions, undesired high molecular weight products were also obtained through the intermolecular transesterification of **8**. All the synthetic dyads **7−9** were purified with gel permeation or flash column chromatography (GPC or FCC) and recrystallization, and characterized by visible absorption, ^{1}H NMR and fast atomic bombardment mass (FAB-MS) spectral analyses.



Scheme 1. Synthesis of cyclic dyad **9**; i) collidine, reflux; ii) $\mathrm{OsO_4}$−$\mathrm{NaIO_4}$/aq. AcOH/THF; iii) conc. HCl; iv) $t\mathrm{BuNH_2BH_3}$/$\mathrm{CH_2Cl_2}$; v) EDC-DMAP/$\mathrm{CH_2Cl_2}$; vi) CMPI-DMAP/toluene, reflux

In the present hetero-dyads, two different chlorin macrocycles are defined as follows: a "pyro"-chlorin moiety lacking the 13^2-ester is called macrocycle "*A*", and the other possessing the 13^2-ester is "*B*" (see the lower portion of Scheme 1 and Figure 1). Additionally, we apply the facial nomenclature at the 13-keto carbonyl group to the facial asymmetry of the chlorin π-plane: the *si*-face of a chlorin moiety is the same as the *si*-face of the C=O plane and the *re*-face of the chlorin is the reverse as shown in Figure 1.

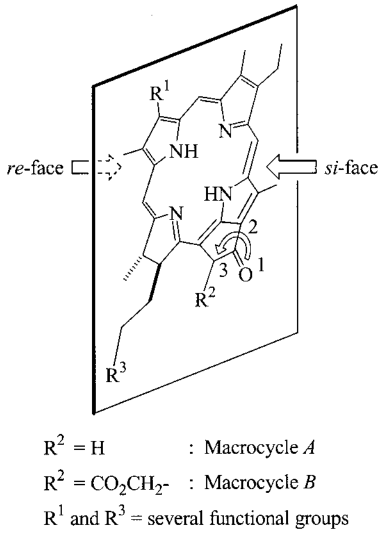


Figure 1. Definition the of chlorin macrocycle and the facial discrimination

Intramolecular cyclization of **8** was a key step in the preparation of the corresponding cyclic dyad **9**. Since transesterification occurs specifically for a β-keto ester with an alcohol, no cleavage was observed at the other esters, *A*-17^2-$\mathrm{CO_2CH_2}$-3-*B*, the linkage between the macrocycles *A* and *B*, and *B*-17^2-$\mathrm{CO_2Me}$ in **8** and **9**. It is also worth noting that the *B*-13^2-$\mathrm{CO_2Me}$ group was unreactive under the standard EDC-DMAP coupling of an acid with an alcohol as in **4** + **6** → **7**.

The absolute configuration at the *B*-13^2-position and the stacking manner between the two macrocycles in the resulting cyclic product **9** were considered, and four isomeric dyads are proposed in Figure 2. These energy-minimized molecular structures were obtained by MM+ and PM3 calculations.[19−21] The side-view and top-view structures are drawn showing the macrocycle *A* plane perpendicular and parallel to the page face, respectively. In the top view, the Q_y transition dipole moments of macrocycles *A* and *B* are depicted by arrows.

In the major *B*-13^2R-epimer of **8**, the activated *B*-13^2-ester could be intramolecularly attacked by the *A*-3^1-OH group from the direction of the *re*- or *si*- face of macrocycle A to the *re*-face of macrocycle *B*, giving isomers **I** and **II**, respectively. On the other hand, in the minor *B*-13^2S-epimer of **8**, the similar cyclization from the direction of the *si*- or *re*-face of macrocycle A to the *si*-face of macrocycle *B*, gave isomers **III** and **IV**, respectively. GPC and NMR spectroscopic analyses of the cyclization mixture showed that a single stereoisomer was obtained as an isolated dyad, indicating that product **9** is one of the four isomers **I−IV**.

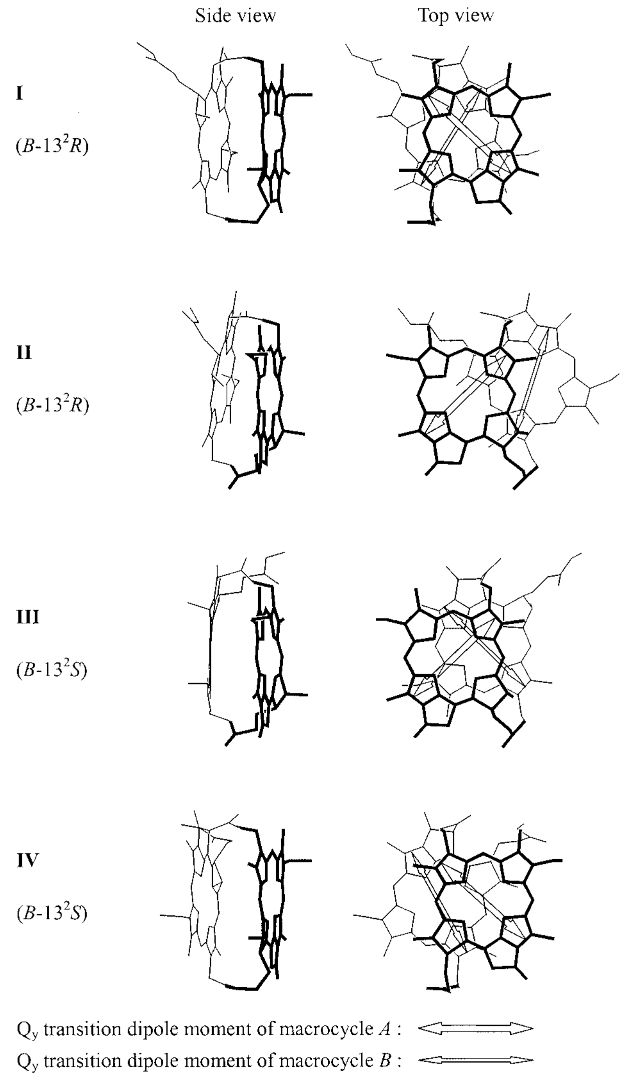


Figure 2. Four possible energy minimized stereostructures **I**−**IV** of cyclic dyads for the coupling product **9**

The following two-dimensional ^1H NMR spectral analyses were useful for the stereochemical determination of the isolated cyclic dyad **9**: the data were collected at 100 °C with a mixing time of 0.15 s in $Cl_2DC-CDCl_2$, since less resolved and broadened signals were obtained at room temperature. The ^1H-^1H ROESY spectrum around the B-13^2-position reveals that the correlation between B-13^2-H and B-17^1-H is stronger than that between B-13^2-H and B-17-H (see the upper-right portion of Figure 3), indicating that 13^2-H is closer to 17^1-H than 17-H in macrocycle B: 13^2-H is situated in the "*syn*"-configuration to 17-CH_2 and in the "*anti*" configuration to 17-H. The relationship is consistent with the R-configuration at the B-13^2-position in **9**. Since no epimerization at the B-13^2 position of **1** occurred under the same conditions as those for the intramolecular esterification, the isolated yield (37%) in the cyclization of a mixture of 13^2R-**8** (ca. 86%) and 13^2S-**8** (ca. 14%) also supports the finding of an R-configuration at B-13^2. As a result, the two isomers, **III** and **IV**, which possessed an S-configuration at the B-13^2-position in **9** were ruled. Isomers **I** and **II**, with an R-configuration remained as the candidates.

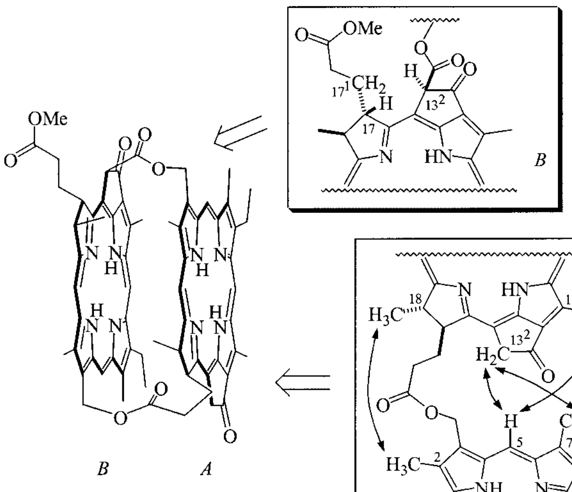
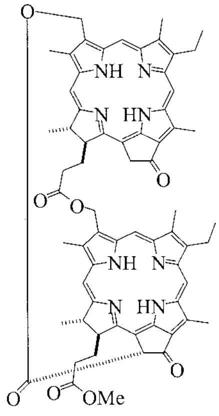


Figure 3. Specific observed NOE correlations of synthetic cyclic chlorophyll hetero-dyad **9** (right) and confirmed stereostructure of **9** (left)

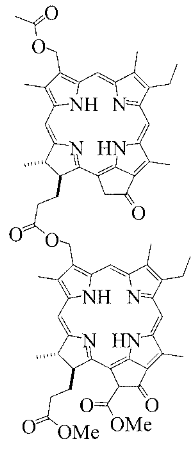
Significant NOE correlations between specific protons on the A and B macrocycles in **9** were observed at A-12-CH_3 ⇄ B-5-H, A-13^2-H ⇄ B-5-H/7-CH_3, and A-18-CH_3 ⇄ B-2-CH_3 (see the lower-right portion of Figure 3). In isomer **I**, all the distances between the above protons are estimated to be 3.7−6.3 Å, which are shorter than those of **II** (7.1−12.4 Å). Isomer **I** is thus determined to be isolated cyclic dyad **9**.

Additionally, the total energies obtained by molecular modeling support the above stereochemical determination. The energies of isomers **I**−**IV** were −31, −18, −21, and −20 kcal·mol^{-1}, respectively, while those of the B-$13^2R/S$-epimers of **8** were −3 and −1 kcal·mol^{-1}, respectively, and that of methanol was 2 kcal·mol^{-1}. The energetic differences ΔE that occur when intramolecular transesterification takes place [= E(isomeric **9**) + E(methanol) − E(epimeric **8**)] were estimated from the above values and ΔE(**I**) was −26 kcal·mol^{-1} which is lower than the other ΔE values, −13 for **II**, −18 for **III**, and −20 kcal·mol^{-1} for **IV**. The largest difference, obtained for the process **8** → isomer **I**, indicates that isomer **I** has the highest thermodynamic stability among them. The results from the molecular modeling study also support that isomer **I** would be readily formed in the intramolecular cyclization from a thermodynamic aspect. Therefore, in the B-13^2R-epimer of **8**, the activated B-13^2-ester by CMPI-DMAP was intramolecularly attacked by the A-3^1-OH from the direction of the *re*-face of macrocycle A to the *re*-face of macrocycle B, stereospecifically giving isomer **I** as the cyclic hetero-dyad **9**.

We prepared a single-linked linear dyad **10** (see the top-right portion of Figure 4) as a reference compound for cyclic dyad **9** through the esterification of the A-3-hydroxymethyl group in **8** with acetic acid. In dyad **10**, there are ester functionalities on both of the 3-positions of macrocycles *A* and *B*, as in **9**. UV/visible absorption and circular dichroism (CD) spectra of cyclic dyad **9** in CH_2Cl_2 were compared with those of the linear dyad **10** (Figure 4). The Q_y absorption maximum of **9** (664 nm) was the same as that of **10**. The Q_y and Soret bands of **9** were slightly broader in the red and the blue regions, respectively, relative to those of **10**. The broadening in **9** (diluted solution, ca. 5 μM) can be ascribed to some interaction of the two chlorin macrocycles in a molecule. Intense S-shaped and reversed S-shaped CD signals in the Q_y and Soret regions, respectively, of **9** demonstrated that the two macrocycles in **9** are close enough to give rise to an intramolecular transition dipole-dipole moment interaction. By applying the CD exciton chirality method,[22] the S-shaped signal observed in the Q_y region of **9** reveals a "clockwise" rotational orientation of the two Q_y transition dipole moments on the N21−N23 axis. This configuration is seen in isomers **I** and **IV** (see Figure 2). This analysis also supports the above determination of isomer **I** from the proposed isomers **I** and **II** with the *B*-13^2*R* configuration.



9



10

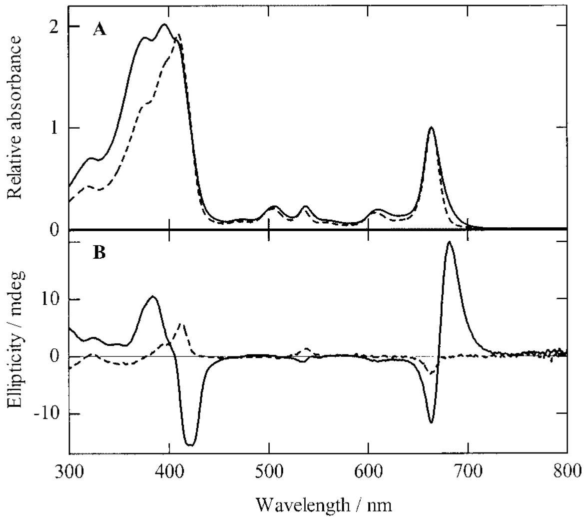


Figure 4. Visible spectra normalized at the Q_y absorption intensity (**A**) and the corresponding CD spectra (**B**) in a CH_2Cl_2 solution of cyclic dyad **9** (solid line) and linear dyad **10** (broken line)

Conclusion

In summary, we synthesized for the first time a cyclic chlorophyll hetero-dyad as a model for the naturally occurring stacked structures of chlorophylls in a photosynthetic system. The present synthetic strategy has great potential for the systematic preparation of a series of cyclic chlorophyll dyads: EDC-DMAP esterification of a formyl-pyrochlorophyllide (as in **4**) with a hydroxy-chlorophyll (as in **6**) and successive reduction of the formyl group of the resulting linear dyad (as in **7**), followed by CMPI-DMAP transesterification of the 13^2-CO_2Me group of the chlorophyll moiety with the hydroxymethyl group of the pyrochlorophyll moiety in the molecule gives the desired cyclic hetero-dyad (as in **9**).

Experimental Section

General: EDC·HCl and DMAP for the coupling of the pigments, and CH_2Cl_2 (analytical grade) for spectroscopic measurements were purchased from Nacalai tesque. *t*$BuNH_2BH_3$ and CMPI were purchased from the Aldrich Chemical Company and Tokyo Chemical Industry, respectively. Toluene, CH_2Cl_2, and $CHCl_3$, used for the syntheses, were commercially available as normal grade and used without further purification. GPC was carried out with recycling preparative high-performance liquid chromatography LC-908 (Japan analytical industry) with three connected polystyrene packed columns (Jaigel 1H, 2H, 3H). FCC was carried out on silica gel (Merck, Kieselgel 60, 9385 or Wako, Wakogel C-300). FAB-MS were recorded on a JEOL GCmate II spectrometer. FAB-MS samples were dissolved in CH_2Cl_2, and polyethylene glycol was used as the internal reference and *m*-nitrobenzyl alcohol and glycerol were used as the matrix. ^1H NMR spectroscopic measurements were performed on a JEOL JNM-A-400 spectrometer, and an internal standard in $CDCl_3$ or $Cl_2DC-CDCl_2$, $CHCl_3$ signal at δ = 7.26 ppm and $Cl_2DC-CDCl_2$ signal at δ = 5.93 ppm, respectively. ^{13}C NMR measurements were performed on a JEOL JNM-ECA-600 spectrometer, and an internal standard in $CDCl_3$ or $Cl_2DC-CDCl_2$, $^{13}CDCl_3$ signal at δ = 77.0 ppm and $Cl_2D^{13}C-CDCl_2$ signal at δ = 75.5 ppm, respectively. UV/visible absorption and CD spectra were measured in air-saturated CH_2Cl_2 at room temperature on a Hitachi U-3500 spectrophotometer and Jasco J-720 W spectropolarimeter, respectively.

Synthesis: All synthetic procedures were performed in the dark. Methyl pyropheophorbide-*d* (**3**)[11,15] and methyl 3-devinyl-3-(hydroxymethyl)pheophorbide-*a*/*a*′ (**6**)[16] were prepared according to reported procedures.

Pyropheophorbide-*d* (4): A concentrated aqueous HCl solution (20 mL) of methyl ester **3** (30 mg, 54 μmol) was stirred at room temperature. After stirring for 3 h, the reaction mixture was poured into ice water and extracted with $CHCl_3$. The aqueous phase was re-extracted with several portions of $CHCl_3$. The combined $CHCl_3$ phases were washed with aqueous $KHSO_4$ (5%) and H_2O, dried over Na_2SO_4, and the solvents were evaporated to dryness. The residue was purified by recrystallization from $CHCl_3$/hexane to give carboxylic acid **4** (24 mg, 45 μmol; 83% yield). Vis (CH_2Cl_2) λ_{max} = 694 (relative intensity, 76%), 668 (14), 633 (9), 554 (16), 522 (15), 426 (100), 388 nm (90). ^1H NMR ($CDCl_3$, 22 °C): δ = 11.51 (s, 1 H, 3-CHO), 10.26 (s, 1 H, 5-H), 9.57 (s, 1 H, 10-H), 8.82 (s, 1 H, 20-H), 5.33, 5.18 (d, $J_{H,H}$ = 20.0 Hz, 1 H each, 13^1-CH_2), 4.57 (br.

q, $J_{H,H}$ = 7.4 Hz, 1 H, 18-H), 4.40 (br. d, $J_{H,H}$ = 9.2 Hz, 1 H, 17-H), 3.75 (s, 3 H, 2-CH_3), 3.69 (q, $J_{H,H}$ = 7.6 Hz, 2 H, 8-CH_2), 3.68 (s, 3 H, 12-CH_3), 3.28 (s, 3 H, 7-CH_3), 2.8−2.7, 2.3−2.2 (m, 1 H each, 17-CH_2), 2.7−2.6, 2.4−2.3 (m, 1 H each, 17^1-CH_2), 1.85 (d, $J_{H,H}$ = 7.4 Hz, 3 H, 18-CH_3), 1.69 (t, $J_{H,H}$ = 7.6 Hz, 3 H, 8^1-CH_3), 0.88, −2.09 ppm (s, 1 H each, NH). MS (FAB) found: *m/z* = 537.2479. Calcd. for $C_{32}H_{33}N_4O_4$: MH^+, 537.2502.

[(Methyl 3-devinylpheophorbide-*a*)-3-yl]methyl Pyropheophorbide-*d* (7): EDC·HCl (90 mg, 469 µmol) and DMAP (160 mg, 1.31 mmol) were added to a CH_2Cl_2 solution (30 mL) of carboxylic acid **4** (60 mg, 112 µmol). Alcohol **6** (67 mg, 110 µmol) was added at 0 °C under an N_2 atmosphere. After stirring for 15 h, the reaction mixture was warmed to room temperature, washed with aqueous HCl (2%), aqueous $NaHCO_3$ (4%) and H_2O, dried with Na_2SO_4, and the solvents were evaporated to dryness. The residue was purified by GPC with $CHCl_3$ and recrystallized from CH_2Cl_2/hexane to give ca. a 1:6 *B*-13^2*R*/*S* mixture of the linear dyad **7** (111 mg, 98.3 µmol; 89% yield). Vis (CH_2Cl_2) λ_{max} = 694 (rel., 35), 665 (39), 638 (7), 608 (7), 555 (9), 533 (10), 507 (10), 410 nm (100). ^1H NMR ($CDCl_3$, 22 °C): δ = 11.29 (s, 1 H, *A*-3-CHO), 10.02 (s, 1 H, *A*-5-H), 9.44 (s, 1 H, *A*-10-H), 9.37 (s, 1 H, *B*-10-H), 9.10 (s, 1 H, *B*-5-H), 8.532, 8.526 (s, 1 H each, *A*- and *B*-20-H), 6.27/6.16 (s, 1 H, *B*-13^1-CH-*R*/*S*), 6.26, 6.09 (d, $J_{H,H}$ = 13.0 Hz, 1 H each, *B*-3-CH_2), 5.31, 5.05 (d, $J_{H,H}$ = 19.6 Hz, 1 H each, *A*-13^1-CH_2), 4.5−4.4 (m, 2 H, *A*- and *B*-18-H), 4.34 (br. d, $J_{H,H}$ = 8.4 Hz, 1 H, *A*-17-H), 4.20 (br. d, $J_{H,H}$ = 8.4 Hz, 1 H, *B*-17-H), 3.88 (s, 3 H, *B*-13^2-CO_2CH_3), 3.66 (s, 3 H, *A*-12-CH_3), 3.64 (s, 3 H, *B*-12-CH_3), 3.57 (q, $J_{H,H}$ = 7.6 Hz, 2 H, *A*-8-CH_2), 3.56 (s, 3 H, 17^2-CO_2CH_3), 3.44 (s, 3 H, *A*-2-CH_3), 3.39 (q, $J_{H,H}$ = 7.6 Hz, 2 H, *B*-8-CH_2), 3.28 (s, 3 H, *B*-2-CH_3), 3.12 (s, 3 H, *A*-7-CH_3), 2.85 (s, 3 H, *B*-7-CH_3), 2.81, 2.45 (m, 1 H each, *A*-17-CH_2), 2.72, 2.42 (m, 1 H each, *A*-17^1-CH_2), 2.64, 2.30 (m, 1 H each, *B*-17-CH_2), 2.53, 2.25 (m, 1 H each, *B*-17^1-CH_2), 1.79 (d, $J_{H,H}$ = 7.2 Hz, 3 H, *B*-18-CH_3), 1.65 (t, $J_{H,H}$ = 7.6 Hz, 3 H, *A*-8^1-CH_3), 1.64 (d, $J_{H,H}$ = 7.6 Hz, 3 H, *A*-18-CH_3), 1.51 (t, $J_{H,H}$ = 7.6 Hz, 3 H, *B*-8^1-CH_3), 0.18, −0.31, −2.00, −2.32 ppm (s, 1 H each, *A*- and *B*-NH). MS (FAB) found: *m/z* = 1129.5256. Calcd. for $C_{67}H_{69}N_8O_9$: MH^+, 1129.5188.

[(Methyl 3-devinylpheophorbide-*a*)-3-yl]methyl 3-Devinyl-3-(hydroxymethyl)pyropheophorbide-*a* (8): Aldehyde **7** (260 mg, 230 µmol) was dissolved in CH_2Cl_2 (40 mL) and $tBuNH_2BH_3$ (20 mg, 230 µmol) was added to the solution while stirring at 0 °C under an N_2 atmosphere. After stirring for 15 h, the reaction mixture was warmed to room temperature, washed with aqueous HCl (2%), aqueous $NaHCO_3$ (4%) and H_2O, dried with Na_2SO_4, and the solvents were evaporated to dryness. The residue was purified by FCC with 1.2% CH_3OH/CH_2Cl_2 (v/v) and recrystallized from CH_2Cl_2/hexane to give a ca. 1:6 *B*-13^2*R*/*S* mixture of alcohol **8** (228 mg, 202 µmol; 88% yield). Vis (CH_2Cl_2) λ_{max} = 663 (rel., 50), 606 (8), 535 (9), 504 (10), 410 nm (100). ^1H NMR ($CDCl_3$, 22 °C): δ = 9.32 (s, 1 H, *B*-10-H), 9.31 (s, 1 H, *A*-10-H), 9.11 (s, 1 H, *A*-5-H), 9.05 (s, 1 H, *B*-5-H), 8.50 (s, 1 H, *B*-20-H), 8.26 (s, 1 H, *A*-20-H), 6.25/6.19 (s, 1 H, *B*-13^1-CH-*R*/*S*), 6.11, 5.90 (d, $J_{H,H}$ = 13.2 Hz, 1 H each, *B*-3-CH_2), 5.55 (br. s, 2 H, *A*-3-CH_2), 5.19, 4.95 (d, $J_{H,H}$ = 19.6 Hz, 1 H each, *A*-13^1-CH_2), 4.44 (br. q, $J_{H,H}$ = 7.6 Hz, 1 H, *B*-18-H), 4.35 (br. q, $J_{H,H}$ = 7.2 Hz, 1 H, *A*-18-H), 4.21 (br. d, $J_{H,H}$ = 9.6 Hz, 1 H, *A*-17-H), 4.19 (br. d, *J* = 8.8 Hz, 1 H, *B*-17-H), 3.89 (s, 3 H, *B*-13^2-CO_2CH_3), 3.62 (s, 3 H, *B*-12-CH_3), 3.56 (s, 6 H, *B*-17^2-CO_2CH_3 and *A*-12-CH_3), 3.5−3.6 (m, 2 H, *A*-8-CH_2), 3.36 (q, $J_{H,H}$ = 7.6 Hz, 2 H, *B*-8-CH_2), 3.21 (s, 3 H, *B*-2-CH_3), 3.05 (s, 3 H, *A*-7-CH_3), 3.03 (s, 3 H, *A*-2-CH_3), 2.88 (s, 3 H, *B*-7-CH_3), 2.70, 2.38 (m, 1 H each, *A*-17^1-CH_2), 2.63, 2.34 (m, 1 H each, *A*-17-CH_2), 2.60, 2.32 (m, 1 H each, *B*-17^1-CH_2), 2.52, 2.23 (m, 1 H each, *B*-17-CH_2), 1.93 (br. s, 1 H, *A*-3^1-OH), 1.78 (d, $J_{H,H}$ = 7.6 Hz, 3 H, *B*-18-CH_3), 1.61 (d, $J_{H,H}$ = 7.2 Hz, 3 H, *A*-18-CH_3), 1.60 (t, $J_{H,H}$ = 7.2 Hz, 3 H, *A*-8^1-CH_3), 1.51 (t, $J_{H,H}$ = 7.6 Hz, 3 H, *B*-8^1-CH_3), 0.19, 0.12, −1.98, −2.04 (s, 1 H each, *A* and *B*-NH) ppm. MS (FAB) found: *m/z* = 1131.5328. Calcd. for $C_{67}H_{71}N_8O_9$: MH^+, 1131.5344.

Cyclic Dyad 9: A toluene solution (10 mL) of **8** (20.0 mg, 17.7 µmol) was added to CMPI (100 mg, 391 µmol) and DMAP (100 mg, 819 µmol) toluene (400 mL) under reflux. After refluxing for 4 h under N_2, the reaction mixture was cooled to room temperature, washed with aqueous saturated Na_2CO_3 and brine, dried over Na_2SO_4, and the solvents were evaporated to dryness. The residue was purified by FCC with 0.6% CH_3OH/CH_2Cl_2 (v/v) and recrystallized from CH_2Cl_2/hexane to give cyclic dyad **9** (7.2 mg, 6.6 µmol; 37% yield). Vis (CH_2Cl_2) λ_{max} = 664 (rel., 50), 610 (9), 537 (11), 506 (11), 396 (100), 377 nm (94). ^1H NMR ($Cl_2CD-CDCl_2$, 100 °C): δ = 9.44 (s, 1 H, *A*-10-H), 9.32 (s, 1 H, *B*-5-H), 8.92 (s, 1 H, *A*-5-H), 8.72 (br. s, 1 H, *B*-10-H), 7.46 (s, 1 H, *B*-20-H), 6.79, 5.40 (d, $J_{H,H}$ = 12.6 Hz, 1 H each, *A*-3-CH_2), 6.64, 6.19 (d, $J_{H,H}$ = 12.4 Hz, 1 H each, *B*-3-CH_2), 6.08 (s, 1 H, B-13^1-CH-(*R*)), 5.72 (br. s, 1 H, *A*-20-H), 5.05 (s, 2 H, *A*-13^1-CH_2), 3.77 (br. q, $J_{H,H}$ = 7.6 Hz, 2 H, *A*-8-CH_2), 3.70 (s, 3 H, *A*-12-CH_3), 3.67 (m, 1 H, *B*-17-H), 3.55, 3.52 (s, 3 H each, *B*-12-CH_3, *B*-17^2-CO_2CH_3), 3.47 (q, $J_{H,H}$ = 7.6 Hz, 2 H, *B*-8-CH_2), 3.33 (s, 3 H, *A*-7-CH_3), 3.19 (s, 3 H, *B*-7- CH_3), 3.06 (m, 1 H, *A*-17-H), 2.98 (s, 3 H, *B*-2-CH_3), 2.93, 1.78 (m, 1 H each, *A*-17^1-CH_2), 2.86 (m, 1 H, *A*-18-H), 2.76, 1.80 (m, 1 H each, *A*-17-CH_2), 2.47, 2.11 (m, 1 H each, *B*-17-CH_2), 2.23, 2.01 (m, 1 H each, *B*-17^1-CH_2), 1.85 (t, $J_{H,H}$ = 7.6 Hz, 3 H, *A*-8^1-CH_3), 1.69 (m, 1 H, *B*-18-H), 1.55 (br. t, $J_{H,H}$ = 7.6 Hz, 3 H, *B*-8^1-CH_3), 0.844 (br. d, $J_{H,H}$ = 7.2 Hz, 3 H, *A*-18-CH_3), 0.835 (br. s, 3 H, *A*-2-CH_3), −0.29 (br. s, 3 H, *B*-18-CH_3), −0.06, −1.20, −3.09, −3.80 ppm (br. s, 1 H each, *A*- and *B*-NH). ^{13}C NMR ($Cl_2DC-CDCl_2$, 100 °C): δ = 196.9, 191.4, 174.5, 174.4, 172.8, 170.0, 169.7, 162.8, 160.6, 156.2, 155.8, 152.2, 151.6, 150.7, 150.2, 146.3, 145.8, 141.8, 139.9, 139.4, 139.1, 137.7, 137.1, 137.1, 136.8, 136.1, 135.1, 134.3, 132.1, 131.9, 131.6, 130.4, 129.3, 106.8, 105.9, 105.1, 104.8, 98.2, 97.2, 93.9, 91.6, 66.8, 59.3, 58.1, 53.2, 52.9, 51.7, 50.8, 50.3, 50.1, 35.1, 32.6, 31.4, 30.6, 22.8, 21.0, 20.8, 20.7, 18.8, 18.5, 13.4, 13.1, 12.8, 12.6, 12.5, 10.3 ppm. MS (FAB) found: *m/z* = 1099.5044. Calcd. for $C_{66}H_{67}N_8O_8$: MH^+, 1099.5082.

[(Methyl 3-devinylpheophorbide-*a*)-3-yl]methyl 3-Devinyl-3-acetoxymethylpyropheophorbide-*a* (10): EDC·HCl (38 mg, 198 µmol) and DMAP (61 mg, 499 µmol) were added to a CH_2Cl_2 solution (30 mL) of acetic acid (10 µL, 175 µmol). Alcohol **8** (20 mg, 17.7 µmol) was added while stirring at 0 °C under an N_2 atmosphere. After stirring for 15 h, the reaction mixture was warmed to room temperature, washed with aqueous HCl (2%), aqueous $NaHCO_3$ (4%) and H_2O, dried with Na_2SO_4, and the solvents were evaporated to dryness. The residue was purified by FCC with 0.6% CH_3OH/CH_2Cl_2 (v/v) and recrystallized from CH_2Cl_2/hexane to give acetyl ester **10** (16 mg, 13.6 µmol; 77% yield). Vis (CH_2Cl_2) λ_{max} = 664 (rel., 51), 608 (8), 535 (9), 505 (11), 410 nm (100). ^1H NMR ($CDCl_3$, 22 °C): δ = 9.42 (s, 2 H, *A*- and *B*-10-H), 9.26 (s, 1 H, *A*-5-H), 9.19 (s, 1 H, *B*-5-H), 8.55 (s, 1 H, *B*-20-H), 8.40 (s, 1 H, *A*-20-H), 6.26, 6.15 (d, $J_{H,H}$ = 13.0 Hz, 1 H each, *B*-3-CH_2), 6.26/6.16 (s, 1 H, *B*-13^1-CH-*R*/*S*), 5.25, 5.00 (d, $J_{H,H}$ = 19.6 Hz, 1 H each, *A*-13^1-CH_2), 4.45 (br. q, $J_{H,H}$ = 7.6 Hz, 1 H, *B*-18-H), 4.40 (br. q, $J_{H,H}$ = 7.6 Hz, 1 H, *A*-18-H), 4.27 (br. d, $J_{H,H}$ = 8.4 Hz, 1 H, *A*-17-H), 4.20 (br. d, *J* = 8.8 Hz, 1 H, *B*-17-H), 3.89 (s, 3 H, *B*-13^2-CO_2CH_3), 3.66 (s, 3 H, *A*-12-CH_3), 3.61 (s, 3 H, *B*-12-CH_3), 3.60 (q, $J_{H,H}$ = 7.6 Hz, 2 H, *A*-8-CH_2), 3.55 (s, 3 H, *B*-17^2-CO_2CH_3), 3.47 (q, $J_{H,H}$ = 7.6 Hz, 2 H, *B*-8-CH_2), 3.31 (s, 3 H, *B*-

2-CH$_3$), 3.22 (s, 3 H, *A*-2-CH$_3$), 3.18 (s, 3 H, *A*-7-CH$_3$), 2.95 (s, 3 H, *B*-7-CH$_3$), 2.77, 2.40 (m, 1 H each, *A*-17^1-CH$_2$), 2.65, 2.36 (m, 1 H each, *A*-17-CH$_2$), 2.63, 2.27 (m, 1 H each, *B*-17^1-CH$_2$), 2.51, 2.22 (m, 1 H each, *B*-17-CH$_2$), 2.12 (s, 3 H, *A*-3^1-OCOCH$_3$), 1.79 (d, $J_{H,H}$ = 7.6 Hz, 3 H, *B*-18-CH$_3$), 1.65 (t, $J_{H,H}$ = 7.6 Hz, 3 H, *A*-8^1-CH$_3$), 1.64 (d, $J_{H,H}$ = 7.6 Hz, 3 H, *A*-18-CH$_3$), 1.55 (t, $J_{H,H}$ = 7.6 Hz, 3 H, *B*-8^1-CH$_3$), 0.24, 0.17, −1.91, −1.97 ppm (s, 1 H each, *A*- and *B*-NH). ^{13}C NMR (CDCl$_3$, 22 °C): δ = 196.0, 189.5, 173.3, 173.0, 172.0, 171.0, 170.99, 169.5, 161.2, 160.0, 155.2, 155.0, 151.1, 151.0, 149.4, 148.7, 145.1, 145.0, 141.1, 140.6, 138.0, 137.9, 136.3, 136.2, 136.0, 135.8, 135.5, 135.1, 132.0, 131.8, 130.6, 129.3, 129.1, 128.5, 106.2, 105.4, 104.2, 103.9, 97.1, 96.7, 93.6, 93.2, 64.7, 57.0, 56.9, 52.9, 51.63, 51.58, 51.1, 50.0, 49.8, 48.0, 31.02, 30.98, 29.8, 29.7, 23.1, 23.0, 21.0, 19.3, 19.2, 17.4, 17.3, 12.1, 12.0, 11.19, 11.15, 11.1, 11.0 ppm. MS (FAB) found: *m/z* = 1173.5461. Calcd. for $C_{69}H_{73}N_8O_{10}$: MH$^+$, 1173.5450.

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