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Synthesis of 3/8-carbonylated chlorophyll derivatives and regiodependent reductivity of their carbonyl substituents

Hitoshi Tamiaki *, Kazunori Hamada, Michio Kunieda

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

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ABSTRACT

Methyl pyropheophorbide-*d* possessing a formyl group at the 3-position and its regioisomer having 8-CHO were prepared and their reactivities with a reductant were determined by the ^{1}H NMR technique: 3-CHO>8-CHO. The regioselective reduction of a synthetic 3,8-diformyl-chlorin also supported the higher reactivity in 3-CHO than in 8-CHO. Regiodependent reduction of the corresponding acetyl-chlorins confirmed that carbonyl groups at the 3-position in chlorophyllous pigments were reduced more rapidly than those at the 8-position. From the reports that reactions of 3-CHO with amines were preferable to those of 7-CHO, the C=O functional groups on the pyrrole A-ring of chlorophylls are more reactive than those on the B-ring.

1. Introduction

All naturally occurring chlorophylls have oxygen-functional groups in a molecule.[1] For example, chlorophyll(Chl)-*a* possesses one keto-carbonyl group at the 13-position and two ester residues at the 13^2- and 17^2-position (see the left of Fig. 1). The other carbonyl groups are found as the peripheral functional groups directly conjugating to the chlorophyllous π-system: 3-CHO in Chl-*d* (see the left of Fig. 1), 3-COMe in bacteriochlorophyll(BChl)-*a* and BChl-*b*, 7-CHO in Chl-*b* and BChl-*e*, and 7-COOMe in Chl-c_3.[2] Such formyl and acetyl groups are so reactive that their alteration was useful for in vitro preparation of modified chlorophylls having different optical and electronic properties from those of the original chlorophylls.[3,4] Moreover, biosynthetic reduction of the formyl group at the 7-position in Chl-*b* to the corresponding hydroxymethyl group has been observed in the course of its interconversion to Chl-*a* possessing the 7-methyl group.[5]

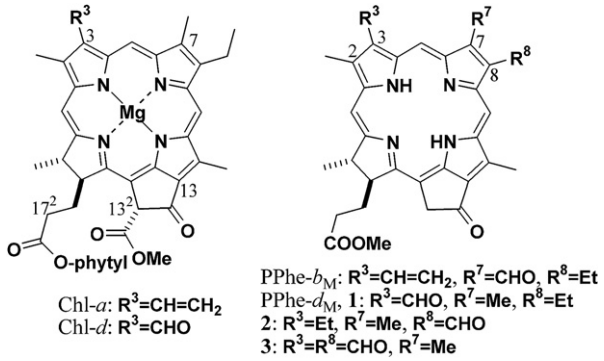

Figure 1. Molecular structures of natural chlorophylls (left) and their synthetic formylated derivatives (right).

The formyl group in natural chlorophylls was conjugated with the chlorin π-system at the 3- or 7-position and affected the visible absorption spectra. The reactivities of the 3- and 7-CHO are assumed to be different and some model systems are available. The 3-CHO of methyl pyropheophorbide-*d* (PPhe-d_M, **1**, see the right of Fig. 1) reacted with amines (RNH_2) to give 3-CH_2NHR and the reductive amination was reported to occur more rapidly than that in 7-CHO of PPhe-b_M.[6] The condensation of 3-CHO in zinc complex of **1** with an amine to afford the corresponding imine was performed eight times more quickly than that of 7-CHO in Zn-PPhe-b_M.[7] The difference in reactivities of 3- and 7-CHO is ascribable to a substituent effect on the asymmetric chlorophyllous macrocyclic π-system. An electronic factor would control the observed reactivity (3-CHO>7-CHO) and the steric factor cannot be ruled out because the substituents near the 3-CHO (2-methyl and 7-methyl groups) are sterically less hindered than those near the 7-CHO (8-ethyl and 3-vinyl groups).

Here, 8-formyl-chlorin **2** (see the right of Fig. 1) was prepared whose formyl group was situated at the same steric environment as

* Corresponding author. Fax: +81 77 561 2659.
E-mail address: tamiaki@se.ritsumei.ac.jp (H. Tamiaki).

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the 3-CHO in **1**; both their first and second neighbor substituents are methyl groups. The reduction of **1** and **2** to hydroxymethyl-chlorins was examined and their difference was discussed in consideration of regioselective reduction of synthetic 3,8-diformyl-chlorin **3**. Moreover, acetyl-chlorins corresponding to formyl-chlorins **1**–**3** were synthesized and their reductions were similarly investigated.

2. Results and discussion

2.1. Synthesis of formylated chlorophyll derivatives 1–3

One of the 3-formyl-chlorins, methyl pyropheophorbide-*d* (**1**) was prepared according to reported procedures.[8] Briefly, naturally occurring Chl-*a* was modified to methyl pyropheophorbide-*a* and the 3-vinyl group was oxidatively cleaved (see step (i) of Scheme 1) to give the corresponding 3-formyl compound **1**.

The isomeric 8-formyl-chlorin **2** was similarly prepared from oxidation of the 8-vinyl compound **9**. Compound **9** was reported in our previous literature[9] and the access was briefly noted. First, selective hydrogenation of the 3-vinyl group in methyl pyropheophorbide-*a* to the ethyl group was performed in acetone which disturbed the undesired reduction of the 13-carbonyl group (see step (ii) of Scheme 1). The reactive C7═C8 double bond of the resulting methyl mesopyropheophorbide-*a* was oxidized to give *cis*-diol **19** (step (iii) of Scheme 1) and successive double dehydration afforded **9** (step (iv) of Scheme 1).

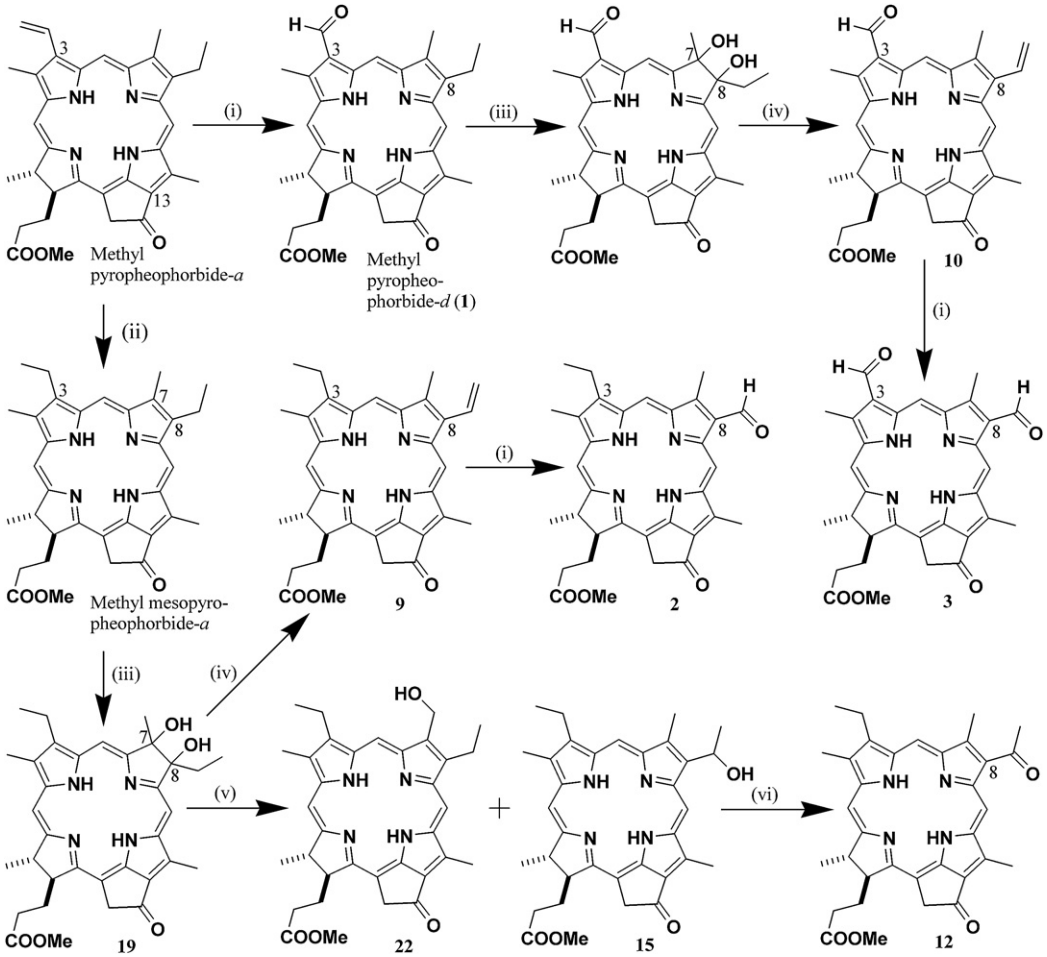
3,8-Diformyl-chlorin **3** was prepared from 3-formyl-8-ethyl-chlorin **1** by the same procedures of 3,8-diethyl-chlorin to 3-ethyl-8-formyl-chlorin **2** mentioned above (see Scheme 1). The isolated yield of the final oxidation of **10** to **3** (step (i)) was 54% and slightly lower than that of **9** to **2** (68%). The decrease would be due to the 3-substituent effect: the electron-withdrawing formyl group at the 3-position suppressed the oxidation of the 8-vinyl group. This effect was pronounced in the double dehydration of 7,8-*cis*-diols to 8-vinyl-chlorins through cationic species (step (iv)): the yield to 3-formylated **10** (24%) was less than half of that to 3-ethylated **9** (60%).[10]

2.2. Synthesis of acetylated chlorophyll derivatives 11–13

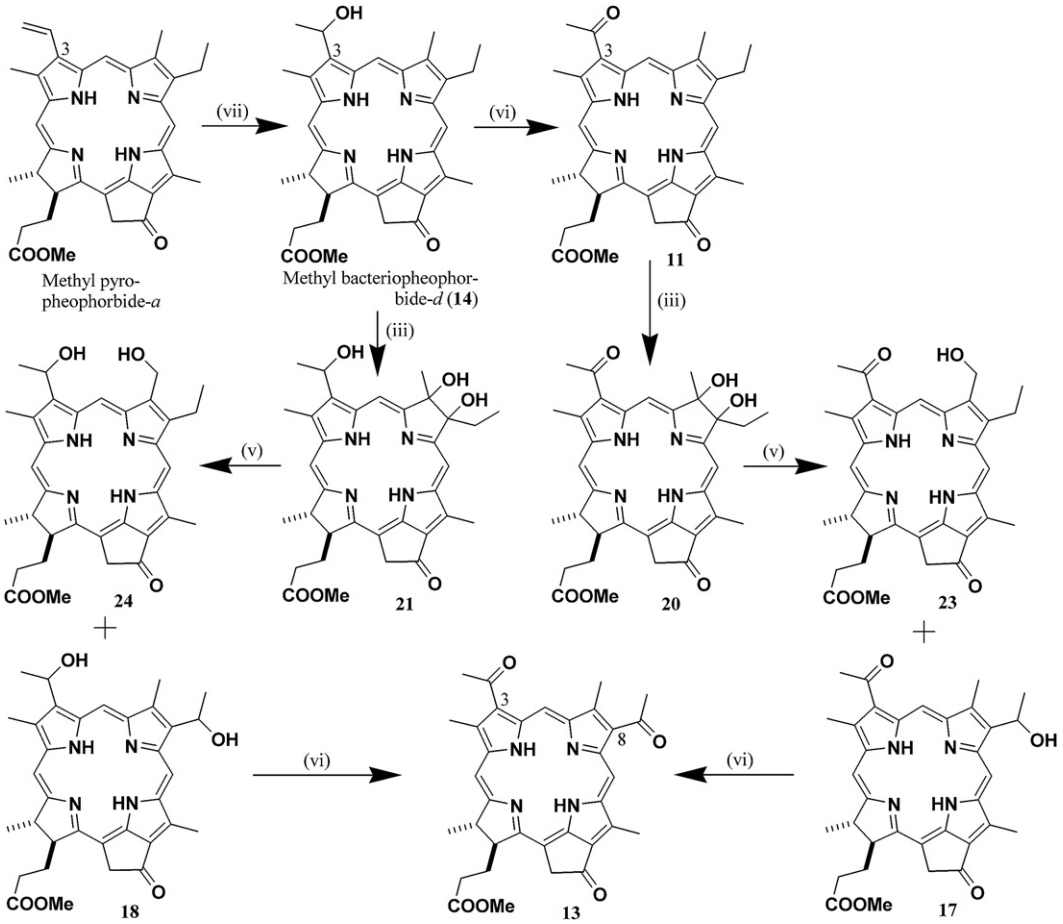
As shown in the upper portion of Scheme 2, 3-acetyl-chlorin **11** was prepared by hydration of the 3-vinyl group of methyl pyropheophorbide-*a* (step (vii)) and oxidation of the 3-(1-hydroxyethyl) group of the resulting methyl bacteriopheophorbide-*d* (**14**) (step (vi)).[11]

8-Acetyl-chlorin **12** isomeric to **11** was obtained as follows (see the lower portion of Scheme 1). Mono-dehydration of *cis*-diol **19** gave a mixture of primary alcohol **22** and secondary alcohol **15** (step (v)). The acid-catalyzed dehydration proceeded more favorably through secondary cationic species as in 8-CH^{+}Me than primary 7-CH_2^{+} species,[12] to afford **15** (50%) more effectively than **22** (18%). The isomeric mixture of alcohols was readily separated by silica gel column chromatography. The major separated product **15** was oxidized to give desired **12** (step (vi)).

By the same procedures as in methyl mesopyropheophorbide-*a* → **19** → **15** → **12** possessing the 3-ethyl group, 3-acetyl-chlorin **11**



Scheme 1. Synthesis of 3/8-formyl-, 3,8-diformyl-, and 8-acetyl-chlorophyll derivatives, **1**/**2**, **3**, and **12**: (i) OsO_4–$NaIO_4$/THF–aq AcOH; (ii) H_2/Pd–C/Me_2CO; (iii) OsO_4/C_5H_5N–CH_2Cl_2, H_2S/MeOH; (iv) *p*TsOH/CH_2Cl_2–C_6H_6 (rt to reflux); (v) aq HCl/$O(CH_2CH_2)_2O$ (50 °C), FCC separation; (vi) Pr_4RuO_4–$O(CH_2CH_2)_2N(O)Me$/CH_2Cl_2.



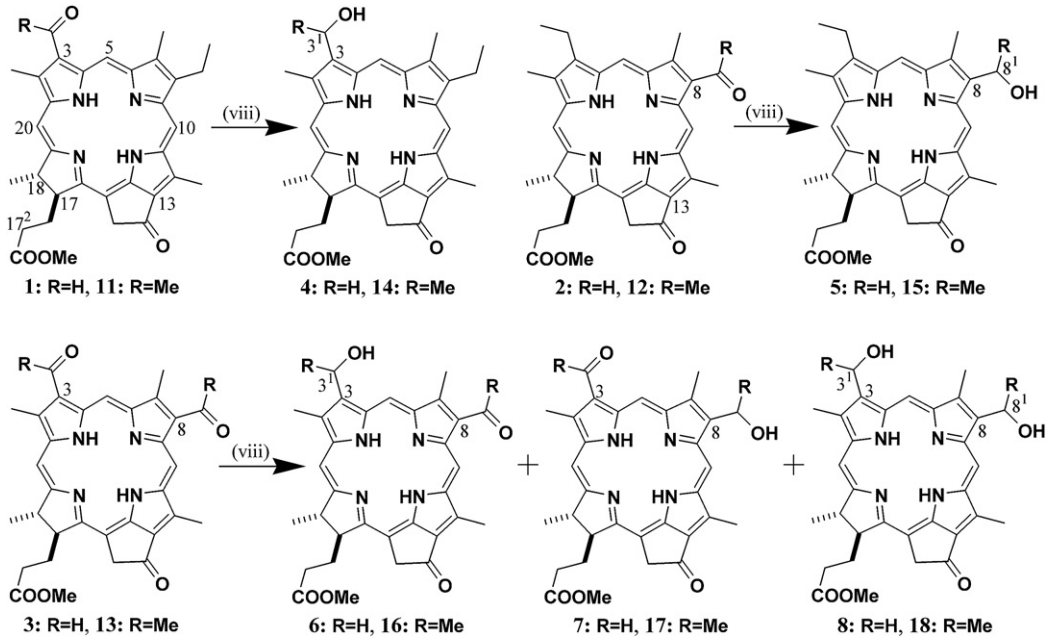
Scheme 2. Synthesis of 3-acetyl- and 3,8-diacetyl-chlorophyll derivatives, **11** and **13**: (iii) $OsO_4/C_5H_5N–CH_2Cl_2$, H_2S/MeOH; (v) aq HCl/$O(CH_2CH_2)_2O$ (50 °C), FCC separation; (vi) $Pr_4RuO_4–O(CH_2CH_2)_2N(O)Me/CH_2Cl_2$; (vii) HBr/AcOH, H_2O, CH_2N_2/Et_2O.

was converted to 3,8-diacetyl-chlorin **13** through **20** and **17** (see the right half of Scheme 2). Alternatively, **13** was prepared from **14** via **21** and **18** (see the left of Scheme 2).[12] The latter route was shorter by one step compared to the former: double one-pot oxidation of **18** to **13** was done in the final stage of the latter, while two oxidations of **14**→**11** and **17**→**13** were needed in the initial and final stages of the former. In spite of there being fewer steps in the latter route, the former access to **13** was effective, because **17** was easily separated from **23** in the isomeric mixture of alcoholic products and separation of **18** from **24** was troublesome.[12] This difference in separation of isomeric alcohols is ascribable to polarities of the molecules: diols **18** and **24** are more polar and interacted more strongly with silica gel than mono-ols **17** and **23**, thus the separation of **18** from **24** was more difficult than that of **17** from **23**.

2.3. Reduction of 3/8-carbonylated chlorophyll derivatives to the corresponding alcohols

3-Formyl-chlorin **1** in chloroform was treated with *tert*-butyl amine borane complex at room temperature to afford 3-hydroxymethyl-chlorin **4** (see Scheme 3).[8] The reduction was clean and compound **4** was exclusively isolated as product just after disappearance of **1**. In the molecule of **1** are three carbonyl groups at 3-, 13-, and 17^2-position and the 3-formyl group was the most reactive as expected: –CHO>–COR>–COOR. 8-Formyl-chlorin **2** was similarly reduced to give the corresponding 8-hydroxymethyl-chlorin **5**. In the initial stage of the reduction, compound **5** was solely obtained as the product and reduction of the 13-keto-carbonyl group was observed in the latter. After consuming **2** completely, desired compound **5** was the main product but the reaction mixture was complex compared with reduction of **1** to **4**. By similar reduction of 3,8-diformyl-chlorin **3**, a single mono-ol and diol **8** were obtained as isolated products. The molecular structure of the isolated mono-ol was determined by ^{1}H NMR and visible absorption spectra to be regioisomeric 3-hydroxymethyl-8-formyl-chlorin **6**. The visible spectrum was especially useful for the determination because the redmost band of chlorins is sensitive to the 3-substituent and is much lesser affected by the 8-functional group.[4,13] The peak position of the resulting mono-ol in dichloromethane was situated at 662 nm which was the same as that of **4** (3-CH_2OH) and blue-shifted compared with 695 nm of **1** (3-CHO), indicating that the mono-ol was **6** possessing the 3-hydroxymethyl group. It is noteworthy that mono-ol **7** isomeric to **6** could not be detected in the reaction mixture from the reduction of **3**.

In similar procedures to the reduction of formyl-chlorins (vide supra), 3-acetyl-chlorin **11** was reduced to 3-(1-hydroxyethyl)-chlorin **14**. Since an acetyl group is less reactive than a formyl group, a prolonged reaction time was necessary for conversion of **11** to **14** and a large amount of the reducing reagent was useful for rapid completion of the reduction. Product **14** has a chiral center at the 3^1-position and was a 1:1 epimeric mixture due to achiral conditions using sole $Me_3CNH_2BH_3$ as a reducing reagent without any chiral additives.[4,14] The remote chiral induction from 17*S* and 18*S* asymmetric carbon atoms could not be observed and product **14** was a diastereomeric mixture. 8-Acetyl-chlorin **12** was reduced to an equimolar 8^1-epimeric mixture of **15** similarly with **11**→**14** and the reduced **15** was predominantly observed as the product in the initial stage. Reduction of 3,8-diacetyl-chlorin **13** gave mono-ol



Scheme 3. Reduction of 3/8-carbonylated chlorophyll derivatives **1**–**3**/**11**–**13** to the corresponding alcohols **4**–**8**/**14**–**18**: (viii) $Me_3CNH_2BH_3/CHCl_3$.

16 and diol **18** and the isomeric mono-ol **17** was not observed. Both the secondary alcoholic products **16** and **18** were epimeric mixtures at 3^1- and 3^1,8^1-position, indicating no asymmetric reduction occurred as expected.

2.4. Regiodependent reductivity of 3/8-carbonyl substituent(s)

The difference in reductivity between the 3- and 8-formyl groups was confirmed by NMR techniques. An equimolar solution of 3-formyl-chlorin **1** and 8-formyl-chlorin **2** in deuterated chloroform was prepared in an NMR sample tube. To the solution was added a $CDCl_3$ solution of $Me_3CNH_2BH_3$, and the ^1H NMR spectra were measured at room temperature. The amounts of each formyl-chlorin were determined by peak areas of low-field shifted protons, 5-, 10-, 20-H and CHO. 3-Formyl-chlorin **1** decreased rapidly and was fully converted to **4** within 10 min (see open circles of Fig. 2A). In contrast, 8-formyl-chlorin **2** was consumed slowly and about half the amount still remained after incubation for 30 min (see closed squares of Fig. 2A). The 3-formyl group was thus more reductive than the 8-formyl group. Compared with the initial slopes of curves in Figure 2A, the reduction rate *k* of **1** to **4** was about five times faster than that of **2** to **5**. The slow reduction of the 8-formyl group in **2** led to the undesired reduction of its 13-carbonyl group during the prolonged reaction time (vide supra).

In the reduction of 3,8-diformyl-chlorin **3**, 3-hydroxymethyl-chlorins **6** and **8** but no 3-formyl-chlorin **7** were isolated as reduced products. This indicated also that the 3-CHO of **3** was more reactive in the reduction than its 8-CHO. The doubly reduced product **8** was prepared by slow reduction of 8-CHO in major **6** and quick reduction of 3-CHO in minor **7**. In the latter case, the second reduction of **7** to **8** (3-CHO→3-CH_2OH) was more easily achieved than the first reduction of **3** to **7** (8-CHO→8-CH_2OH), and **7** could not be detected in the reaction mixture.

Under the same conditions as the reduction of formyl-chlorins **1** and **2** with $Me_3CNH_2BH_3$ in $CDCl_3$, an equimolar solution of 3- and 8-acetyl-chlorins **11** and **12** was examined and their consumed amounts were determined at intervals by ^1H NMR spectroscopy. The acetyl group at the 3-position of **11** was reduced about 10 times more slowly than the 3-CHO of **1** but was quantitatively transferred to the 1-hydroxyethyl group in **14** within 2 h (see Fig. 2B). The 8-COMe of **12** was reduced to about five times slower in the initial stage than the 3-COMe of **11**. The relative suppression of *k*-values in reduction of **12**→**15** over that of **11**→**14** was the same as in formyl-chlorins, $k_{\mathbf{2}\rightarrow\mathbf{5}}/k_{\mathbf{1}\rightarrow\mathbf{4}}$=ca. 1:5. The difference in reductivity of 3- and 8-acetyl-chlorins **11** and **12** was supported by the selective reduction of 3,8-diacetyl-chlorin **13** at which products **16** and **18** reduced at the 3-acetyl group were obtained, but no 3-acetyl-chlorins including **17** were isolated as reduction products from the reaction mixture.

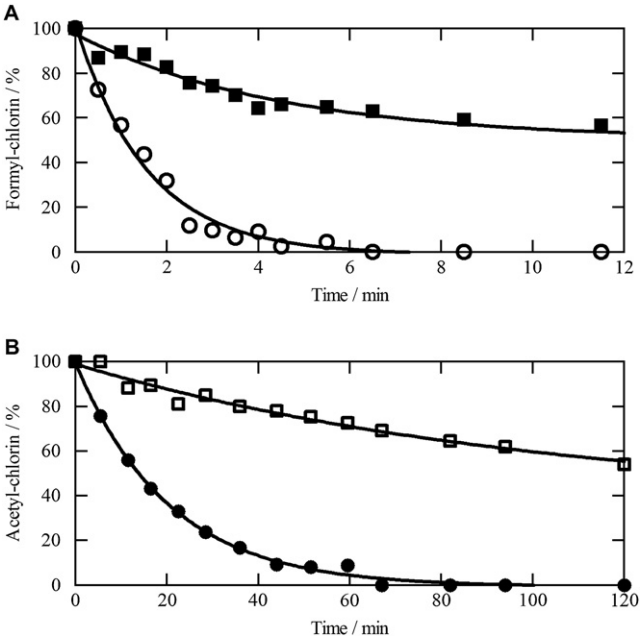


Figure 2. Consumption of 3-formyl-chlorin **1** (open circles) and 8-formyl-chlorin **2** (closed squares) (**A**, upper) and that of 3-acetyl-chlorin **11** (closed circles) and 8-acetyl-chlorin **12** (open squares) (**B**, lower) by reduction with $Me_3CNH_2BH_3$ in $CDCl_3$.

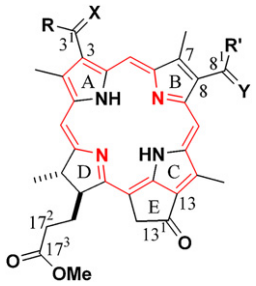


Figure 3. A major 18π-circuit (red) of chlorophyll derivatives.

Table 1
Stretching vibrational peaks ν (cm^{-1})[a] of carbonyl groups of 3- and 8-carbonyl-chlorins in CH_2Cl_2

Compound	ν(3-C=O)	ν(8-C=O)	ν(13-C=O)	ν(17^2-C=O)
1 (3-CHO)	1677		1697	1734
2 (8-CHO)		1663	1697	1734
11 (3-COMe)	1671		1695	1734
12 (8-COMe)		1657	1696	1734

[a] Errors were within 1 cm^{-1}.

2.5. The reduction reactivity of 3-C=O > 8-C=O

As mentioned above, the 3-carbonyl group of chlorin chromophores is more reactive with a reducing agent than the 8-carbonyl group. The difference is explained by consideration of a chlorin π-conjugated system. The major aromatic 18π-circuit of a chlorin chromophore is drawn by the red line of Figure 3.[15] The 3-C=O is attached on the largely delocalized 18π-system and the 8-C=O is bonded with the relatively isolated double bond C7=C8, giving that the conjugation of the 3-C=O with the 18π-system would be weaker than that of the 8-C=O with C7=C8. Therefore, the 3-C=O is more isolated from the adjacent π-system than the 8-C=O, and the former is a more reactive carbonyl substituent than the latter. This explanation was confirmed by their FTIR and ^{13}C NMR spectra as follows.

In dichloromethane, the stretching vibrational band of the 3-formyl carbonyl group in **1** was observed at ν=1677 cm^{-1} and ν of the 8-formyl carbonyl group in **2** was 1663 cm^{-1} (see Table 1). The lower wavenumber shift ($\Delta\nu$=14 cm^{-1}) in **1**→**2** showed that the peripheral 3-CHO was less conjugated with the adjacent π-system than the 8-CHO. In acetyl-chlorins **11** and **12**, the same shift was observed [$\Delta\nu$=1671 (for **11**)−1657 (for **12**)=14 cm^{-1}] and less conjugation of 3-COMe than 8-COMe was ascertained.

In chloroform-*d*, the chemical shift δ of 3-formyl carbon atom in **1** was 188.14 ppm and the δ of $C8^1$ in **2** was 187.15 ppm (see Table 2). The low-field shift in **2**→**1** indicated that the $C3^1$ of **1** was more deshielded and electropositive than the $C8^1$ of **2**, and the 3-CHO was more reactive with a nucleophile than the 8-CHO. The similar tendency was observed in acetyl-chlorins **11** and **12**; δ of $C3^1$ in **11** and $C8^1$ in **12** was 199.39 and 198.96, respectively.

Table 2
Chemical shifts δ (ppm)[a] of carbonyl carbon-13 atoms of 3- and 8-carbonyl-chlorins in $CDCl_3$

Compound	δ($C3^1$)	δ($C8^1$)	δ($C13^1$)	δ($C17^3$)
1 (3-CHO)	188.14		195.94	173.36
2 (8-CHO)		187.15	195.77	173.36
11 (3-COMe)	199.39		196.09	173.44
12 (8-COMe)		198.96	196.07	173.44

[a] Errors were within 0.02 ppm.

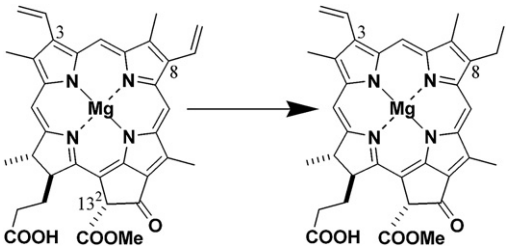


Figure 4. Enzymatic hydrogenation of divinyl-chlorophyllide-*a* to chlorophyllide-*a* in a biosynthetic route to Chl-*a*.

3. Concluding remarks

The present results clearly indicated that the 3-carbonyl group in synthetic chlorophyll derivatives was reduced more smoothly than the 8-carbonyl group. The substituent effect is ascribable to the difference in electronic factor at the substituted positions, not to the steric factor. Considering the reported data that the 3-formyl group was more easily reduced than the 7-formyl group (see Section 1), the carbonyl groups on an A-ring (see Fig. 3) would be more reactive than those on a B-ring. The preferable reduction of the 3-carbonyl group is consistent with the report[16] that the 3-formyl group of Chl-*d* (see Fig. 1) in an aqueous solution was easily altered by treatment of a weak reductant, sodium dithionite.

Recently, Tanaka and his colleagues reported that the 8-vinyl group of divinyl-chlorophyllide-*a* in a biosynthetic route to Chl-*a* was hydrogenated to the 8-ethyl group (see Fig. 4),[17] in spite of the presence of the 3-vinyl group. Such an enzymatic reduction is exclusively performed at the 8-substituent, not at the 3-substituent. This is in sharp contrast with the regiodependent reactivity based on the present reduction of C=O to CHOH. One of the reasons for the reverse selectivity is the high site-recognition of the substrate (divinyl-chlorophyllide-*a*) by the enzyme (divinyl reductase). The other is the difference between regiodependent reactivities in reduction of carbonyl groups in pyrochlorophyll derivatives lacking 13^2-COOMe and hydrogenation of vinyl groups in the chlorophyllide possessing 13^2-COOMe;[10,18] the 8-vinyl group would be hydrogenated more easily than the 3-vinyl group.

4. Experimental

4.1. General

All melting points were measured with a Yanagimoto micro melting apparatus and were uncorrected. Visible absorption spectra were measured with a Hitachi U-3500 spectrophotometer. ^1H NMR spectra in $CDCl_3$ were measured with a Bruker AC-300 (300 MHz) and JEOL ECA-600 (600 MHz) spectrometers at room temperature; chemical shifts (δ) are expressed in parts per million relative to $CHCl_3$ (7.26 ppm) as an internal reference. ^{13}C NMR spectra in $CDCl_3$ were measured with a JEOL ECA-600 (150 MHz) spectrometer; δs are expressed in parts per million relative to $^{13}CDCl_3$ (77.0 ppm) as an internal reference. FABMS spectra were measured with a JEOL GCmate II spectrometer; FABMS samples were dissolved in CH_2Cl_2, *m*-nitrobenzyl alcohol was used as the matrix and PEG600 was added as an external reference for HRMS measurements. FTIR spectra were measured with a Shimadzu FTIR-8600 spectrophotometer; samples in CH_2Cl_2 were measured in a KBr cell.

All synthetic procedures were done in the dark under N_2. THF was distilled from CaH_2 before use and CH_2Cl_2 was distilled without moisture before use. Flash column chromatography (FCC) was performed on silica gel, Kieselgel 60 (Merck, 40–63 μm).

4.2. Materials

Methyl pyropheophorbide-*d* (**1**),[8] methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (**4**),[8] methyl 8-deethyl-8-hydroxymethyl-mesopyropheophorbide-*a* (**5**),[9] methyl 3^1-oxo-mesopyropheophorbide-*a* (**11**),[11] methyl bacteriopheophorbide-*d* (**14**),[19] and 3^1,8^1-dihydroxy-mesopyropheophorbide-*a* (**18**)[12] were prepared according to reported procedures.

4.3. Synthesis of 3/8-carbonylated chlorophyll derivatives

*4.3.1. Methyl 8-deethyl-8-formyl-mesopyropheophorbide-a (**2**)*

To 8-vinyl-chlorin **9** (48.5 mg, 88 μmol)[9] in THF (25.0 ml), OsO_4 (2.3 mg) was added with stirring and cooled to 0 °C. A solution of $NaIO_4$ (48.8 mg) and AcOH (64 μl) in H_2O (1.0 ml) was dropped into the above ice-chilled THF solution, stirred overnight at room temperature, and poured into ice-water. After extraction with CH_2Cl_2 several times, the combined organic phase was washed with aq 4% $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by FCC (5% Et_2O/CH_2Cl_2) and recrystallized from CH_2Cl_2 and hexane to give 8-formyl-chlorin **2** (33.0 mg, 68% yield);[9] black solid; mp 243–246 °C; vis (CH_2Cl_2) λ_{max}=659 (relative intensity, 27), 606 (5), 571 (3), 522 (4), 435 (100), 413 (36) nm; ^{1}H NMR ($CDCl_3$) δ=11.12 (1H, s, CHO), 10.36, 9.31, 8.56 (each 1H, s, 5-, 10-, 20-H), 5.26, 5.11 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.51 (1H, dq, J=2, 7 Hz, 18-H), 4.31 (1H, dt, J=6, 2 Hz, 17-H), 3.85 (2H, q, J=8 Hz, 3-CH_2), 3.64, 3.63, 3.60, 3.26 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.70–2.76, 2.56–2.62, 2.26–2.35 (1H+1H+2H, m, 17-CH_2CH_2), 1.84 (3H, d, J=7 Hz, 18-CH_3), 1.75 (3H, t, J=8 Hz, 3^1-CH_3), −0.05, −1.94 (each 1H, s, NH×2). MS (FAB) found: m/z 550, calcd for $C_{33}H_{34}N_4O_4$: M^+, 550. HRMS (FAB) found: m/z 550.2592, calcd for $C_{33}H_{34}N_4O_4$: M^+, 550.2580.

*4.3.2. Methyl 8-deethyl-8-formyl-pyropheophorbide-d (**3**)*

Similar to the synthesis of **2**, oxidation of 3-formyl-8-vinyl-chlorin **10** (24.0 mg, 44 μmol)[10] by OsO_4 (3.2 mg) and $NaIO_4$ (60.5 mg) in THF (10.0 ml), H_2O (1.0 ml), and AcOH (77 μl) [using $CHCl_3$ as extracted and recrystallized solvents instead of CH_2Cl_2] gave 3,8-diformyl-chlorin **3** (13.0 mg, 54% yield); dark brown solid; mp>300 °C; vis (CH_2Cl_2) λ_{max}=685 (rel, 32), 625 (6), 542 (9), 455 (100) nm; ^{1}H NMR ($CDCl_3$) δ=11.53, 11.18 (each 1H, s, CHO), 10.58, 10.49, 8.88 (each 1H, s, 5-, 10-, 20-H), 5.35, 5.20 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.60 (1H, dq, J=2, 7 Hz, 18-H), 4.41 (1H, dt, J=9, 2 Hz, 17-H), 3.80, 3.74, 3.72, 3.64 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.74–2.79, 2.61–2.66, 2.27–2.38 (1H+1H+2H, m, 17-CH_2CH_2), 1.87 (3H, d, J=7 Hz, 18-CH_3), −0.45, −2.08 (each 1H, s, NH×2). MS (FAB) found: m/z 550, calcd for $C_{32}H_{30}N_4O_5$: M^+, 550. HRMS (FAB) found: m/z 550.2592, calcd for $C_{32}H_{30}N_4O_5$: M^+, 550.2216.

*4.3.3. Methyl 7^1-hydroxy-mesopyropheophorbide-a (**22**) and methyl 8^1-hydroxy-mesopyropheophorbide-a (**15**)*

To 7,8-*cis*-diol **19** (50.0 mg, 86 μmol)[9] in 1,4-dioxane (20 ml), aq concd HCl (40 μl) and H_2O (4 ml) were added. After being stirred for 30 min at 50 °C, the reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The organic phase was washed with aq 4% $NaHCO_3$ and water, and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by FCC (1.0–1.2% $MeOH/CH_2Cl_2$ for **22** and 0.3–0.5% $MeOH/CH_2Cl_2$ for **15**) and recrystallized from CH_2Cl_2 and hexane to give **22** (9.0 mg, 18% yield) and **15** (25.0 mg, 50% yield, $8^1R/S$=1:1). Compound **22**: dark green solid; mp 113–116 °C; vis (CH_2Cl_2) λ_{max}=651 (rel, 35), 597 (7), 538 (6), 506 (8), 414 (100), 397 (66) nm; ^{1}H NMR ($CDCl_3$) δ=9.45, 9.43, 8.46 (each 1H, s, 5-, 10-, 20-H), 5.75 (2H, s, 7-CH_2), 5.19, 5.05 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.44 (1H, dq, J=2, 7 Hz, 18-H), 4.27 (1H, dt, J=8, 2 Hz, 17-H), 3.83, 3.76 (each 2H, q, J=8 Hz, 3-, 8-CH_2), 3.61, 3.57, 3.28 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.63–2.68, 2.51–2.56, 2.19–2.33 (1H+1H+2H, m, 17-CH_2CH_2), 1.79 (3H, d, J=7 Hz, 18-CH_3), 1.73, 1.72 (each 3H, t, J=8 Hz, 3^1-, 8^1-CH_3), 0.47, −1.67 (each 1H, s, NH×2). MS (FAB) found: m/z 566, calcd for $C_{34}H_{38}N_4O_4$: M^+, 566. Compound **15**: black solid; mp 128–130 °C; vis (CH_2Cl_2) λ_{max}=654 (rel, 39), 599 (7), 538 (7), 507 (9), 413 (100), 397 (71) nm; ^{1}H NMR ($CDCl_3$) δ=10.00/9.99, 9.25, 8.48 (each 1H, s, 5-, 10-, 20-H), 6.26 (1H, q, J=7 Hz, 8-CH), 5.24, 5.09 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.47 (1H, dq, J=2, 8 Hz, 18-H), 4.27 (1H, dt, J=9, 2 Hz, 17-H), 3.85 (2H, q, J=8 Hz, 3-CH_2), 3.67, 3.61, 3.36, 3.30 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.66–2.70, 2.53–2.57, 2.25–2.33 (1H+1H+2H, m, 17-CH_2CH_2), 2.14 (3H, d, J=7 Hz, 8^1-CH_3), 1.80 (3H, d, J=8 Hz, 18-CH_3), 1.73 (3H, t, J=8 Hz, 3^1-CH_3), 0.44, −1.73 (each 1H, s, NH×2). MS (FAB) found: m/z 566, calcd for $C_{34}H_{38}N_4O_4$: M^+, 566. HRMS (FAB) found: m/z 566.2879, calcd for $C_{34}H_{38}N_4O_4$: M^+, 566.2893.

*4.3.4. Methyl 8^1-oxo-mesopyropheophorbide-a (**12**)*

To 8-(1-hydroxyethyl)chlorin **15** (17.0 mg, 31 μmol) in CH_2Cl_2 (15 ml), *N*-methylmorphorine *N*-oxide (12.4 mg) and tetra-*n*-propylammonium perruthenate (4.0 mg) were added and stirred for 2 h at room temperature. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic phase was washed with water, and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by FCC (5% Et_2O/CH_2Cl_2) and recrystallized from CH_2Cl_2 and hexane to give **12** (10.0 mg, 59% yield); black solid; mp 98–101 °C; vis (CH_2Cl_2) λ_{max}=656 (rel, 29), 602 (6), 559 (4), 517 (6), 430 (100), 411 (44) nm; ^{1}H NMR ($CDCl_3$) δ=10.18, 9.23, 8.51 (each 1H, s, 5-, 10-, 20-H), 5.21, 5.06 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.47 (1H, dq, J=2, 8 Hz, 18-H), 4.26 (1H, dt, J=9, 2 Hz, 17-H), 3.79 (2H, q, J=8 Hz, 3-CH_2), 3.63, 3.58, 3.49, 3.29, 3.07 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.66–2.72, 2.54–2.60, 2.24–2.33 (1H+1H+2H, m, 17-CH_2CH_2), 1.82 (3H, d, J=8 Hz, 18-CH_3), 1.71 (3H, t, J=8 Hz, 3^1-CH_3), 0.06, −2.00 (each 1H, s, NH×2). MS (FAB) found: m/z 564, calcd for $C_{34}H_{36}N_4O_4$: M^+, 564. HRMS (FAB) found: m/z 564.2737, calcd for $C_{34}H_{36}N_4O_4$: M^+, 564.2735.

*4.3.5. Methyl 7^1-hydroxy-3^1-oxo-mesopyropheophorbide-a (**23**) and methyl 8^1-hydroxy- 3^1-oxo-mesopyropheophorbide-a (**17**)*

Similar to the synthesis of **22**/**15**, dehydration of 7,8-*cis*-diol **20** (10.0 mg, 16 μmol)[4] in 1,4-dioxane (10 ml), aq concd HCl (20 μl), and H_2O (2 ml) gave **23** (1.7 mg, 18% yield) and **17** (3.4 mg, 38% yield, $8^1R/S$=1:1). Compound **23**: black solid; vis (CH_2Cl_2) λ_{max}=678 (rel, 43), 619 (5), 549 (9), 518 (14), 421 (100) nm; ^{1}H NMR ($CDCl_3$) δ=10.15, 9.67, 8.78 (each 1H, s, 5-, 10-, 20-H), 5.81 (2H, s, 7-CH_2), 5.31, 5.21 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.54–4.57 (1H, m, 18-H), 4.34–4.37 (1H, m, 17-H), 3.84 (2H, q, J=8 Hz, 8-CH_2), 3.70, 3.64, 3.62, 3.31 (each 3H, s, 2-, 3^1-, 12-CH_3, $COOCH_3$), 2.57–2.70, 2.27–2.35 (each 2H, m, 17-CH_2CH_2), 1.83 (3H, d, J=7 Hz, 18-CH_3), 1.63 (3H, t, J=8 Hz, 8^1-CH_3), −2.02 (1H, s, NH) [another NH was too broad to be observed]. MS (FAB) found: m/z 580, calcd for $C_{34}H_{38}N_4O_5$: M^+, 580. Compound **17**:[20] black solid; vis (CH_2Cl_2) λ_{max}=680 (rel, 45), 621 (7), 548 (9), 518 (12), 419 (100) nm; ^{1}H NMR ($CDCl_3$) δ=10.16, 10.03, 8.78 (each 1H, s, 5-, 10-, 20-H), 6.26 (1H, q, J=7 Hz, 8-CH), 5.31, 5.21 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.54–4.57 (1H, m, 18-H), 4.34–4.37 (1H, m, 17-H), 3.70, 3.66, 3.61, 3.37, 3.30 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.57–2.70, 2.27–2.35 (each 2H, m, 17-CH_2CH_2), 2.15/2.13 (3H, d, J=7 Hz, 8^1-CH_3), 1.83 (3H, d, J=7 Hz, 18-CH_3), −2.10 (1H, s, NH) [another NH was too broad to be observed]. MS (FAB) found: m/z 580, calcd for $C_{34}H_{38}N_4O_5$: M^+, 580.

*4.3.6. Methyl 3^1,8^1-dioxo-mesopyropheophorbide-a (**13**)*

Similar to the synthesis of **12**, oxidation of 3-acetyl-8-(1-hydroxyethyl)-chlorin **17** gave **13**;[20] black solid; mp 194–197 °C (lit.[20] 198–199 °C); vis (CH_2Cl_2) λ_{max}=675 (rel, 31), 617 (5), 529 (9), 442 (100), 386 (70) nm; ^{1}H NMR ($CDCl_3$) δ=9.85 (1H, s, 10-H), 9.76 (1H, s, 5-H), 8.72 (1H, s, 20-H), 5.21, 5.04 (each 1H, d, J=19 Hz,

13^1-CH_2), 4.54 (1H, dq, J=2, 8 Hz, 18-H), 4.31 (1H, dt, J=10, 2 Hz, 17-H), 3.66 (3H, s, 17-$COOCH_3$), 3.62 (3H, s, 2-CH_3), 3.41 (3H, s, 12-CH_3), 3.27 (3H, s, 7-CH_3), 3.21 (3H, s, 3^1-CH_3), 2.91 (3H, s, 8^1-CH_3), 2.72–2.78, 2.61–2.66, 2.36–2.41, 2.24–2.31 (each 1H, m, 17-CH_2CH_2), 1.89 (3H, d, J=8 Hz, 18-CH_3), −1.15, −2.71 (each 1H, s, NH×2). MS (FAB) found: m/z 578, calcd for $C_{34}H_{34}N_4O_5$: M^+, 578.

4.4. Reduction of carbonyl group

Carbonylated chlorin in $CHCl_3$ was reduced by $Me_3CNH_2BH_3$ (3–5 equiv for aldehyde and >10 equiv for ketone) with stirring at room temperature. The reaction was monitored by visible spectra and/or TLC. After disappearance of the starting chlorin, the reaction mixture was quenched by aq dil HCl at 0 °C, washed with aq 4% $NaHCO_3$ and water, and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by FCC (0.6–0.8% MeOH/CH_2Cl_2 for mono-ol and 1.2–1.5% MeOH/CH_2Cl_2 for diol) and recrystallization (CH_2Cl_2/hexane) to give the corresponding alcohol. All the 1-hydroxyethylated compounds prepared by reduction of acetyl group gave a diastereomeric mixture.

*4.4.1. Methyl 3,8-dediethyl-8-formyl-3-hydroxymethyl-pyropheophorbide-a (**6**)*

Black solid; vis (CH_2Cl_2) λ_{max}=662 (rel, 27), 608 (5) 571 (3), 522 (4), 436 (100), 415 (37) nm; ^{1}H NMR ($CDCl_3$) δ=10.85 (1H, s, CHO), 9.87, 9.36, 8.62 (each 1H, s, 5-, 10-, 20-H), 5.90 (2H, s, 3-CH_2), 5.17, 5.03 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.51 (1H, dq, J=2, 8 Hz, 18-H), 4.27 (1H, dt, J=10, 2 Hz, 17-H), 3.67, 3.46, 3.43, 3.42 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.70–2.75, 2.59–2.65, 2.36–2.40, 2.23–2.29 (each 1H, m, 17-CH_2CH_2), 1.87 (3H, d, J=8 Hz, 18-CH_3), −0.87, −2.41 (each 1H, s, NH×2). MS (FAB) found: m/z 581, calcd for $C_{34}H_{37}N_4O_5$: MH^+, 581.

*4.4.2. Methyl 3,8-dediethyl-3,8-bis(hydroxymethyl)-pyropheophorbide-a (**8**)*

Black solid; mp 148–151 °C; vis (CH_2Cl_2) λ_{max}=660 (rel, 44), 604 (7), 539 (7), 508 (9), 415 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.71, 9.52, 8.60 (each 1H, s, 5-, 10-, 20-H), 5.94, 5.76 (each 2H, s, 3-, 8-CH_2), 5.16, 5.04 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.48 (1H, dq, J=2, 8 Hz, 18-H), 4.24 (1H, dt, J=10, 2 Hz, 17-H), 3.64, 3.63, 3.44, 3.38 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.55–2.65, 2.28–2.32, 2.17–2.24 (2H+1H+1H, m, 17-CH_2CH_2), 1.79 (3H, d, J=8 Hz, 18-CH_3), −0.10, −1.96 (each 1H, s, NH×2). MS (FAB) found: m/z 554, calcd for $C_{32}H_{34}N_4O_5$: M^+, 554.

*4.4.3. Methyl 3^1-hydroxy-8^1-oxo-mesopyropheophorbide-a (**16**)*

Black solid (3^1*R*/*S*=1:1); mp 78–84 °C; vis (CH_2Cl_2) λ_{max}=659 (rel, 29), 604 (5), 559 (3), 517 (5), 431 (100) nm; ^{1}H NMR ($CDCl_3$) δ=10.183/10.179, 9.85/9.82, 8.56/8.54 (each 1H, s, 5-, 10-, 20-H), 6.40–6.45 (1H, m, 3-CH) 5.18/5.13, 5.04/5.01 (each 1H, d, J=19 Hz, 13^1-CH_2), 4.46–4.54 (1H, m, 18-H), 4.20–4.27 (1H, m, 17-H), 3.66/3.63, 3.59, 3.50/3.49, 3.43/3.42, 3.09/3.08 (each 3H, s, 2-, 7-, 8^1-, 12-CH_3, $COOCH_3$), 2.55–2.71, 2.21–2.39 (each 2H, m, 17-CH_2CH_2), 2.15/2.14 (3H, d, J=7 Hz, 3^1-CH_3), 1.83/1.78 (3H, d, J=7 Hz, 18-CH_3), −0.36/0.44, −2.18/2.24 (each 1H, s, NH×2). MS (FAB) found: m/z 553, calcd for $C_{32}H_{33}N_4O_5$: MH^+, 553.

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References and notes

1. Scheer, H. *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications*; Grimm, B., Porra, R. J., Rüdiger, W., Scheer, H., Eds.; Springer: Dordrecht, The Netherlands, 2006; Chapter 1, pp 1–26.
2. Tamiaki, H.; Shibata, R.; Mizoguchi, T. *Photochem. Photobiol.* **2007**, *83*, 152.
3. Johnson, S. G.; Small, G. J.; Johnson, D. G.; Svec, W. A.; Wasielewski, M. R. *J. Phys. Chem.* **1989**, *93*, 5437; Osuka, A.; Marumo, S.; Wada, Y.; Yamazaki, I.; Shirakawa, Y.; Nishimura, Y. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2909; Zheng, G.; Pandey, R. K.; Forsyth, T. P.; Kozyrev, A. N.; Dougherty, T. J.; Smith, K. M. *Tetrahedron Lett.* **1997**, *38*, 2409; Tamiaki, H.; Kouraba, M. *Tetrahedron* **1997**, *53*, 10677; Holzwarth, A. R.; Katterle, M.; Müller, M. G.; Ma, Y.-Z.; Prokhorenko, V. *Pure Appl. Chem.* **2001**, *73*, 469; Yagai, S.; Miyatake, T.; Shimono, Y.; Tamiaki, H. *Photochem. Photobiol.* **2001**, *73*, 153; Li, G.; Dobhal, M. P.; Shibata, M.; Pandey, R. K. *Org. Lett.* **2004**, *6*, 2393; Tamiaki, H.; Kitamoto, H.; Nishikawa, A.; Hibino, T.; Shibata, R. *Bioorg. Med. Chem.* **2004**, *12*, 1657; Sasaki, S.; Kotegawa, Y.; Tamiaki, H. *Tetrahedron Lett.* **2006**, *47*, 4849.
4. Sasaki, S.; Omoda, M.; Tamiaki, H. *J. Photochem. Photobiol., A: Chem.* **2004**, *162*, 307.
5. Tanaka, A.; Tanaka, R. *Curr. Opin. Plant Biol.* **2006**, *9*, 248.
6. Efimov, A.; Tkachenko, N. V.; Vainiotalo, P.; Lemmetyinen, H. *J. Porphyrins Phthalocyanines* **2001**, *5*, 835.
7. Rau, H. K.; Snigula, H.; Struck, A.; Robert, B.; Scheer, H.; Haehnel, W. *Eur. J. Biochem.* **2001**, *268*, 3284.
8. Tamiaki, H.; Amakawa, M.; Shimono, Y.; Tanikaga, R.; Holzwarth, A. R.; Schaffner, K. *Photochem. Photobiol.* **1996**, *63*, 92.
9. Yagai, S.; Miyatake, T.; Tamiaki, H. *J. Photochem. Photobiol., B: Biol.* **1999**, *52*, 74.
10. Kunieda, M.; Mizoguchi, T.; Tamiaki, H. *Tetrahedron* **2004**, *60*, 11349.
11. Tamiaki, H.; Yagai, S.; Miyatake, T. *Bioorg. Med. Chem.* **1998**, *6*, 2171.
12. Tamiaki, H.; Omoda, M.; Saga, Y.; Morishita, H. *Tetrahedron* **2003**, *59*, 4337.
13. Sasaki, S.; Tamiaki, H. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 797; Sasaki, S.; Tamiaki, H. *J. Org. Chem.* **2006**, *71*, 2648.
14. Tamiaki, H.; Kouraba, M.; Takeda, K.; Kondo, S.; Tanikaga, R. *Tetrahedron: Asymmetry* **1998**, *9*, 2101.
15. Steiner, E.; Fowler, P. W. *ChemPhysChem* **2002**, *3*, 114.
16. Tomo, T.; Suzuki, T.; Hirano, E.; Tsuchiya, T.; Miyashita, H.; Dohmae, N.; Mimuro, M. *Chem. Phys. Lett.* **2006**, *423*, 282.
17. Nagata, N.; Tanaka, R.; Tanaka, A. *Plant Cell Physiol.* **2007**, *48*, 1803.
18. Struck, A.; Cmiel, E.; Katheder, I.; Schäfer, W.; Scheer, H. *Biochim. Biophys. Acta* **1992**, *1101*, 321.
19. Tamiaki, H.; Takeuchi, S.; Tsudzuki, S.; Miyatake, T.; Tanikaga, R. *Tetrahedron* **1998**, *54*, 6699.
20. Inhoffen, H. H.; Jäger, P.; Mählhop, R.; Mengler, C.-D. *Liebigs Ann. Chem.* **1967**, *704*, 188.