Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 4337–4350

TETRAHEDRON

Synthesis of homologously pure bacteriochlorophyll-*e* and *f* analogues from BChls-*c*/*d* via transformation of the 7-methyl to formyl group and self-aggregation of synthetic zinc methyl bacteriopheophorbides-*c*/*d*/*e*/*f* in non-polar organic solvent

Hitoshi Tamiaki,* Miki Omoda, Yoshitaka Saga and Hidetada Morishita

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

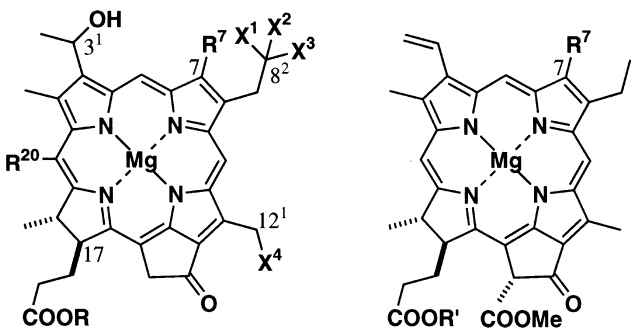
Received 6 March 2003; accepted 11 April 2003

Abstract—Homologously pure methyl bacteriopheophorbides-*e* and *f* (BPhes-*e*/f_M) were prepared from modification of naturally occurring bacteriochlorophylls-*c* and *d* (BChls-*c*/*d*), respectively, by transformation of the methyl to formyl group at the 7-position. The absolute configuration of the 1-hydroxyethyl group at the 3-position of (Zn-)BPhes-*e*/f_M was determined from comparison with structurally known BChl-*c*/*d* epimers. Visible spectra of synthetic (Zn-)BPhe-*c*/*d*/*e*/f_M showed that the 7^1-oxidation and the $8^2/12^1$/20-methylation affected Soret, Q_x and Q_y bands of both the monomeric (in a polar organic solvent) and oligomeric species (in a non-polar solvent).

1. Introduction

Photosynthetic green bacteria are characterized by extra-membraneous light-harvesting antenna apparatuses, called 'chlorosomes'. Chlorosomes are unique peripheral antenna systems where composite chlorophylls self-aggregate to form large oligomers surrounding with a galactolipid monolayer.[1,2] Such a system is completely different from any other peripheral and all integral antennas consisting of pigment–protein complexes. The difference is ascribable to molecular structures of composite chlorophyllous pigments. Self-aggregative chlorophylls in chlorosomes are usually bacteriochlorophylls-*c* and *d* (BChls-*c*/*d*), as shown in the left drawing of Figure 1. As special anoxygenic green sulfur bacteria, brown-colored species are found in deep water under low light conditions, e.g. *Chlorobium phaeobacteroides*.[3] In any brown-colored bacteria, BChl-*e* molecules self-aggregate to form a core part of the chlorosome. BChl-*e* is a magnesium complex of 7-formyl-chlorin and is differentiated by the 7-substituent (R^7) from BChl-*c* which possesses the 7-methyl group. The same molecular relationship is seen in more popular pigments of higher plants between chlorophyll-*b* (Chl-*b*, 7-CHO) and Chl-*a* (7-CH_3) (see the right drawing of Fig. 1). Moreover, the term BChl-*f* is reserved for the 7-formyl derivative of naturally occurring BChl-*d* possessing the 7-methyl group; BChl-*f* has not yet been found in any green bacteria.

BChls-*c*/*d*/*e*(/*f*) are family names of several molecular variants (see the left drawing of Fig. 1): 3^1*R*/*S*-epimers (stereoisomer), $8^2/12^1$-methylated substituents (X^n=H/CH_3, homolog) and several ester chains (R) on the 17-propionate including farnesyl and strearyl groups.[4] The variation is



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
BChl-*f*: R^7=CHO, R^{20}=H
($X^{1\text{-}4}$=H or CH_3;
R=farnesyl, stearyl, etc.)

Chl-*a*: R^7=CH_3
Chl-*b*: R^7CHO
(R'=phytyl)

Figure 1. Molecular structures of naturally occurring chlorophylls.

Keywords: bacteriochlorophyll; photosynthetic green bacteria; self-aggregate; substitution effect; visible spectra.

* Corresponding author. Tel.: +81-77-561-2765; fax: +81-77-561-2659; e-mail: tamiaki@se.ritsumei.ac.jp

0040–4020/03/$ - see front matter
doi:10.1016/S0040-4020(03)00638-0

dependent upon the species as well as the cultured conditions.[2,5,6] Such molecular derivatizations control the efficiency of light absorption and energy transfer in a chlorosome, while the other antennas do it by controlling composite proteins.

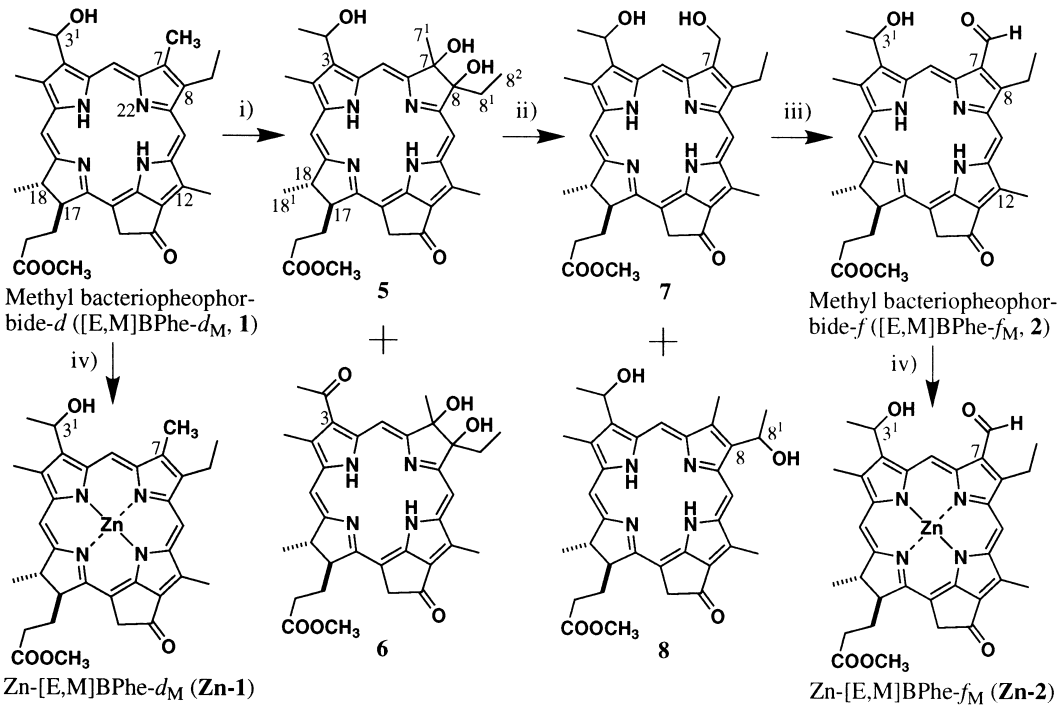
Some methyl groups at the β-position of porphyrins and chlorins have been reported to be oxidized to the formyl groups.[7,8] No reports are available, to our best knowledge, of such an oxidation of the methyl group at the specific position on a chlorophyll π-system. Here we report preparation of homologously pure BChl-*e*/*f* derivatives from chemical modification of naturally occurring BChl-*c*/*d* by transformation of the 7-methyl to 7-formyl group,[9] and visible absorption spectra of their monomers in dichloromethane as well as oligomers of the zinc complexes in 1% (v/v) dichloromethane and cyclohexane.

2. Results and discussion

2.1. Preparation of 7-formyl-chlorins from 7-methyl-chlorins

Methyl 8-ethyl-12-methyl-bacteriopheophorbide-*d* ([E,M]BPhe-d_M, **1**) prepared from modification of chlorophyll-*a*[10] was oxidized by osmium tetraoxide in the presence of pyridine in dichloromethane and successive treatment of the resulting osmate with hydrogen sulfide[7,11] to give the corresponding 7,8-diol **5** after purification of flash column chromatography on silica gel (FCC) and recrystallization from dichloromethane and hexane (see Scheme 1). The 7–8 double bond is the most reactive in the chlorin π-conjugated system[12] and natural enzymes do reduce the double bond to afford 7,8-dihydro-chlorin as in BChl-*a* moiety.[13] The high reactivity is ascribable to the fact that the double bond is less conjugated to the major 18π-system through the nitrogen atom at the 22-position in a chlorin. Under the above oxidation conditions, the secondary alcohol on the 3-position was also oxidized and over-oxidized compound **6**[11] was observed after prolonged reaction. Avoiding use of a large excess of OsO_4 and quenching the reaction mixture with H_2S just after consumption of **1** (checked by TLC) resulted in the predominant formation of **5** in a moderate yield (56%). The NOE correlation between 7^1-H and $8^1/8^2$-H clearly showed that the diol **5** was *cis*-configuration, consistent with the proposed one from *syn*-oxidation by OsO_4, while no NOE between 17-H and 18-H or obvious NOE between 17-H and 18^1-H was observed. The stereochemistry at the 7- and 8-positions was not determined but the *cis*-diol **5** was a 7/6 diastereomeric mixture (7*R*,8*S* or 7*S*,8*R*) from the ^{1}H NMR spectral analysis. The small but present stereoselectivity was due to remote control of the stereochemically determined 17,18-substituents. Such a diastereochemical differentiation would be controlled directly (through space) or indirectly (via the 3^1-hydroxy group).

The resulting *cis*-diol **5** was dissolved in 1,4-dioxane and treated with aqueous diluted hydrogen chloride at 50°C for a few minutes[7] to give mono-dehydrated compounds **7**[14] and **8** after FCC and recrystallization. Such mild dehydration procedures are important for selective preparation of mono-dehydrates. Strong acidic conditions and/or prolonged reaction led to undesired reactions: pinacol–pinacolone rearrangement (to 7-ethyl-7-methyl-8-oxo- and 8-ethyl-8-methyl-7-oxo-forms),[15] double dehydration (to 7-methyl-8-vinyl-chlorin)[11] and additional dehydration on the 3-(1-hydroxyethyl) group (to 3-vinyl-chlorin). The mono-dehydrated product was a regioisomeric mixture of

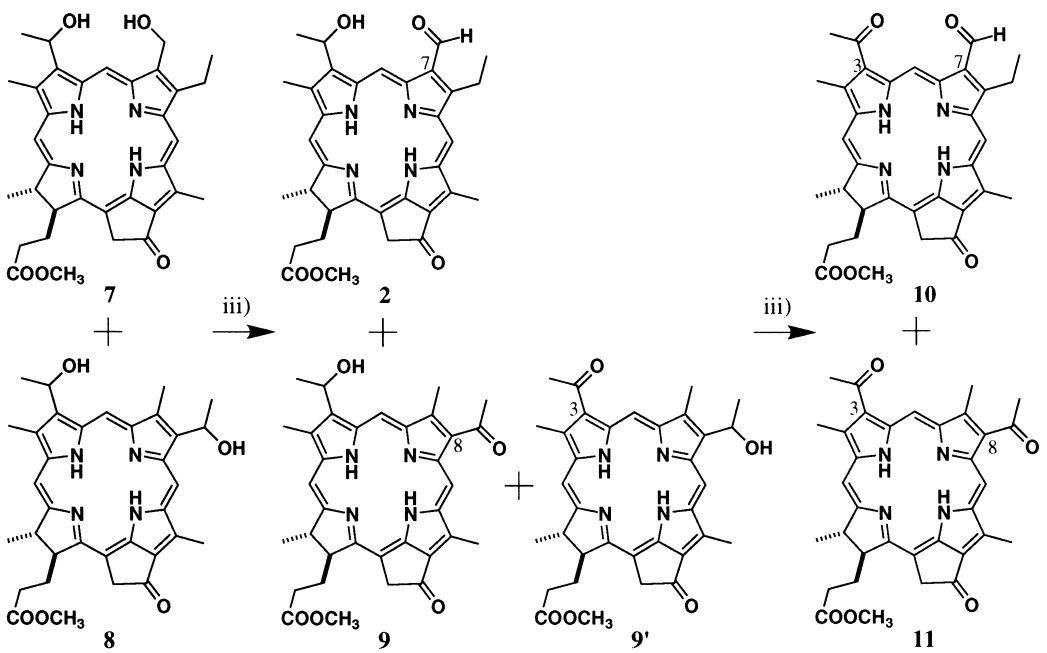


Scheme 1. Synthesis of methyl 8-ethyl-12-methyl-bacteriopheophorbide-*f* ([E,M]BPhe-f_M, **2**) and its zinc complex (Zn-[E,M]BPhe-f_M, Zn-**2**) by modification of the corresponding 7-methyl derivative ([E,M]BPhe-d_M, **1**); (i) OsO_4–C_5H_5N/CH_2Cl_2, H_2S/MeOH; (ii) aq. HCl/$O(CH_2CH_2)_2O$ at 50°C; (iii) PDC/C_6H_6; (iv) $Zn(OAc)_2{\cdot}2H_2O$/MeOH–CH_2Cl_2.

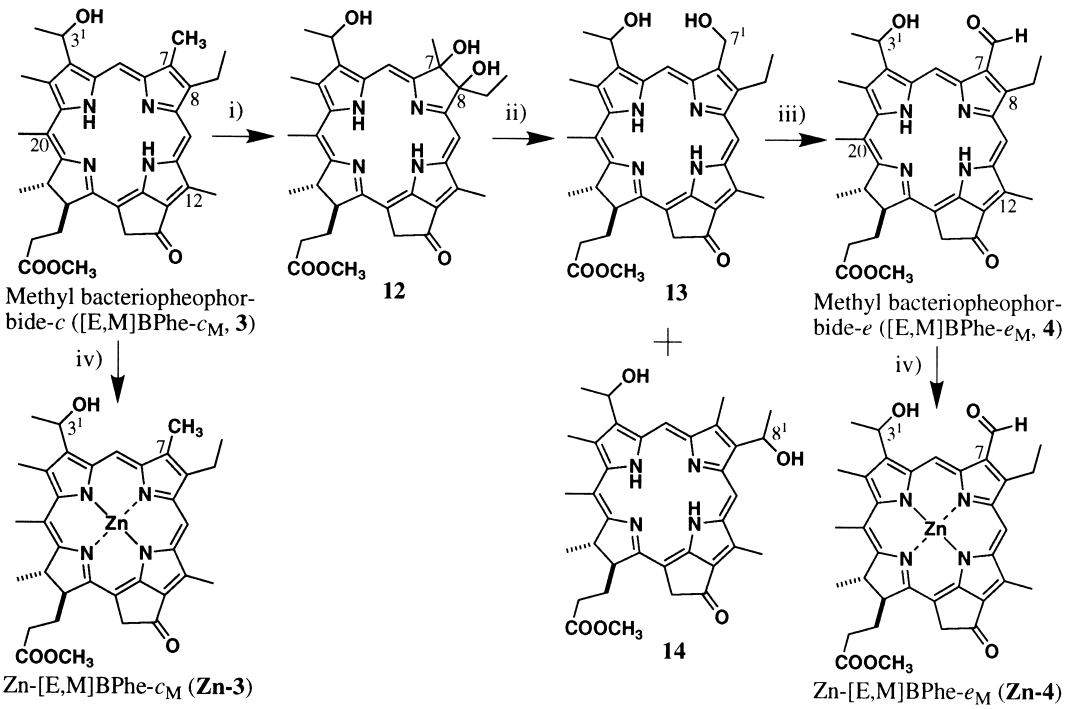
7-(hydroxymethyl)chlorin **7** (10%) and 8-(1-hydroxyethyl)-chlorin **8** (81%). The selectivity would be due to the fact that a secondary cationic intermediate like 8-CH^+CH_3 is more stable than a primary cationic one like 7-CH_2^+. The isomeric mixture was separated by continuous FCC eluted with dichloromethane and methanol to afford less polar secondary alcohol **8** as the first fraction and more polar primary alcohol **7** as the second fraction. To achieve easier separation, selective oxidation of the isomeric mixture was examined by pyridinium dichromate (PDC, vide infra), but the complex mixture was obtained including desired **2** and undesired **9** and **9′** (Scheme 2) because an 8-fold amount of less reactive secondary alcohol **8** was partially oxidized during the oxidation of a lower quantity of more reactive primary alcohol **7**. The reaction mixture was so complex that isolation of pure **2** by FCC was not possible at this stage. Moreover, completely oxidized 3-acetyl-7-formyl-chlorin **10** and 3,8-diacetyl-chlorin **11** could not be separated on FCC or reverse-phase HPLC because their polarity is almost the same. It is noteworthy that the stereochemistry produced at the 8^1-position in the resulting **8** was *R*/*S*=1/1 from the ^{1}H NMR analysis, indicating there was no diastereomeric control in the dehydration.

According to reported procedures,[14] 7-(hydroxymethyl)-chlorin **7** was oxidized by PDC in benzene to give 7-formyl-chlorin **2**, methyl 8-ethyl-12-methyl-bactereiopheophor-bide-*f* ([E,M]BPhe-f_M) in 79% yield. Therefore, the 7-methyl group of **1** was converted to the formyl group of **2** by the above 3 steps. The resulting **2** possesses a chiral center at the 3^1-position. The starting material **1** derived from acidic hydration of 3-vinyl-chlorin, methyl pyropheo-phorbide-*a* was a 1/1 3^1-epimerical mixture,[10] so that product **2** was also a stereochemical mixture. After usual zinc-metallation, the stereoisomers of Zn-**1** were readily separated by reverse-phase HPLC (first eluting 3^1R and second eluting 3^1S)[10] and the separated Zn-**1** was rapidly demetallated by stirring of the dichloromethane solution with aqueous acid to afford epimerically pure **1**. Using 3^1S-rich compound **1** (*R*/*S*=1/9) as the starting material, the above transformation gave 3^1S-rich compound **2** at the same ratio (*R*/*S*=1/9), which was determined by the HPLC analysis[14] after zinc-metallation. The result shows that no epimerization occurred at the 3^1-position during the above transformation procedures from the 7-methyl to formyl group.

Transformation of methyl 8-ethyl-12-methyl-bacteriopheo-phorbide-*c* ([E,M]BPhe-c_M, **3**) possessing the 7-methyl group to methyl 8-ethyl-12-methyl-bacteriopheophorbide-*e* ([E,M]BPhe-e_M, **4**) possessing the 7-formyl group was then examined (see Scheme 3). [E,M]BPhe-c_M (**3**) and e_M (**4**) are 20-methylated forms of [E,M]BPhe-d_M (**1**) and f_M (**2**), respectively. [E,M]BPhe-c_M (**3**) was prepared from modification of [E,M]BChl-*c* extracted from *Chloroflexus aurantiacus* cultured in aqueous media.[14] The extracted [E,M]BChl-*c* was a 2/1 mixture of 3^1R and 3^1S epimers based on the natural abundance and the present [E,M]BPhe-c_M (**3**) was also the 3^1-epimerical mixture at the same ratio. Dihydroxylation of **3** by OsO_4 gave *cis*-diol **12** (67%) which was a 7/6 diastereomeric mixture at the 7,8-positions. Mild acidic dehydration of **12** afforded regioisomers of 7^1-hydroxy-chlorin **13** (10%) and 8^1-hydroxy-chlorin **14** (80%) and the separation on FCC was successful. Finally, selective oxidation of separated primary alcohol **13** afforded aldehyde **4** (52%). These results show that the 20-methylation did not affect the transformation of 7-methyl to formyl group, which is useful for preparation of BPhe-*e* as well as BPhe-*f*. The resulting [E,M]BPhe-e_M (**4**) was a 2/1 3^1-epimerical mixture, indicating that the stereo-chemistry at the 3^1-position was retained through the above procedures as expected; 3^1R-Zn-**4** was eluted more rapidly than 3^1S-Zn-**4** on a reverse-phase HPLC (see also Ref. 10). Synthetic **4** is helpful for determination of 3^1-stereochemistry of [E,M]BChl-*e* which was recently found



Scheme 2. Oxidation of a mixture of 7^1- and 8^1-hydroxy-[E,M]BPhes-d_M, **7** and **8**; (iii) PDC/C_6H_6.



Scheme 3. Synthesis of methyl 8-ethyl-12-methyl-bacteriopheophorbide-*e*([E,M]BPhe-e_M, **4**) and its zinc complex (Zn-[E,M]BPhe-e_M, Zn-**4**) by modification of the corresponding 7-methyl derivative ([E,M]BPhe-c_M, **3**); (i) OsO_4–C_5H_5N/CH_2Cl_2, H_2S/MeOH; (ii) aq. HCl/$O(CH_2CH_2)_2O$ at 50°C; (iii) PDC/C_6H_6; (iv) $Zn(OAc)_2{\cdot}2H_2O$/MeOH–CH_2Cl_2.

as a minor 3^1-epimerically-unknown component in a strain of brown-colored green sulfur bacteria.[16]

2.2. Visible spectra of monomers and self-aggregates of (zinc) methyl bacteriopheophorbides-*c*/*d*/*e*/*f*

In a diluted dichloromethane solution, all four free-base chlorins **1**-**4** gave sharp Q_y (longest wavelength/lowest energy) and Soret bands (at around 400 nm) in the absorption spectra (Fig. 2), indicating they were monomeric. Comparing their bands of **1** with **2** and of **3** with **4**, transformation of the 7-methyl to 7-formyl group implied that the Q_y and Soret peaks move to shorter (ca. 10 nm/230 cm^{-1}) and longer wavelengths (ca. 30 nm/1600 cm^{-1}), respectively, and that the ratio of Q_y/Soret peak intensities decreased to less than half (Table 1). These spectral changes are consistent with observations reported previously in several 7-methyl- and formyl-chlorins,[14] and are ascribable to an electronic effect of the 7-substituent. Substitution with a methyl group at the 20-position as in **1**→**3** and **2**→**4** led to the following shifts. Both the Q_y and Soret peaks were shifted to longer wavelengths by about 10 nm/230 cm^{-1} and 10 nm/460 cm^{-1}, respectively. The bathochromic shifts were also observed in other 20-unsubstituted- and methyl-chlorins,[10] mainly due to sterically induced π-conjugate disturbance. The 20-methyl group slightly suppressed the ratio of Q_y/Soret peaks. The band at a wavelength about 55 nm/1400 cm^{-1} shorter from the Q_y-peak position shows almost the same shifts by 7-CH_3 to CHO and 20-H to CH_3 as the Q_y band does. As a result, the relatively small band is

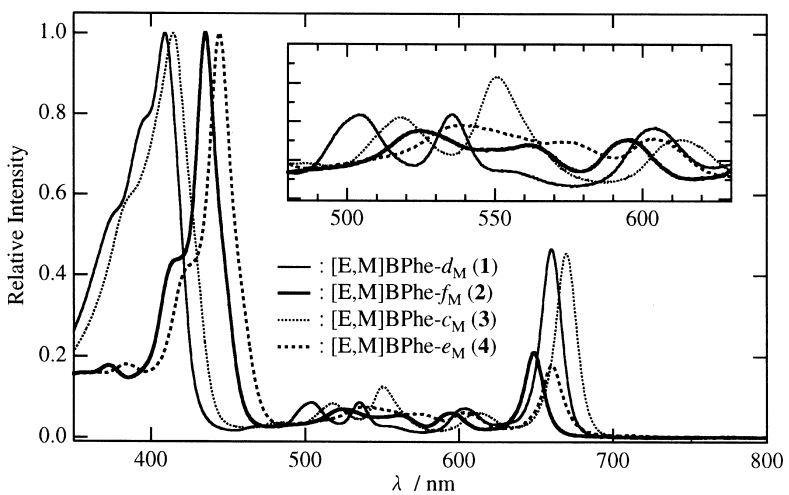


Figure 2. Visible spectra of methyl 8-ethyl-12-methyl-bacteriopheophorbides-*d*/*f*/*c*/*e* ([E,M]BPhes-*d*/*f*/*c*/e_M, **1/2/3/4**) in CH_2Cl_2, normalized at each Soret peak.

Table 1. Absorption maxima (nm) of [E,M]BPhes-x_M **1** (x=*d*; $3^1R/S$=1/1), **2** (x=*f*; $3^1R/S$=1/1), **3** (x=*c*; $3^1R/S$=2/1) and **4** (x=*e*; $3^1R/S$=2/1) in dichloromethane and the relative intensities of their peak heights (in parenthesis)

	Q_y	Q_y'	Q_x	Q_x'	Soret
1 (R^7=CH_3, R^{20}=H)	660 (46) [370][a]	603 (7)	535 (9)	505 (9)	409 (100)
2 (R^7=CHO, R^{20}=H)	649 (21) [350][a]	595 (6)	562 (6)	525 (7)	435 (100)
3 (R^7=CH_3, R^{20}=CH_3)	669 (45) [360][a]	613 (6)	550 (13)	518 (8)	415 (100)
4 (R^7=CHO, R^{20}=CH_3)	660 (18) [370][a]	605 (6)	575 (6)	537 (8)	445 (100)

[a] FWHM of Q_y band (cm^{-1}).

Table 2. Absorption maxima (nm) of Zn-[E,M]BPhes-x_M **1** (x=*d*; $3^1R/S$=1/1), **2** (x=*f*; $3^1R/S$=1/1), **3** (x=*c*; $3^1R/S$=2/1) and **4** (x=*e*; $3^1R/S$=2/1) in dichloromethane and the relative intensities of their peak heights (in parenthesis)

	Q_y	Q_y'	Q_x	Q_x'	Soret
Zn-**1** (R^7=CH_3, R^{20}=H)	647 (76) [390][a]	601 (11)	554 (5)	513 (3)	422 (100)
Zn-**2** (R^7=CHO, R^{20}=H)	632 (35) [490][a]	585 (9)			450 (100)
Zn-**3** (R^7=CH_3, R^{20}=CH_3)	658 (62) [410][a]	612 (11)	575 (6)	527 (4)	427 (100)
Zn-**4** (R^7=CHO, R^{20}=CH_3)	643 (30) [570][a]	593 (9)			457 (100)

[a] FWHM of Q_y band (cm^{-1}).

assigned to a vibrational component of Q_y band, designated Q_y' band here. It is noteworthy that no change was observed in the width of Q_y band by alternation of the above functional groups in the present synthetic free base chlorins; the full widths at half maxima (FWHM) of the Q_y band are almost the same, 360±10 cm^{-1} (Table 1).

The peak positions of the other two bands at the region over 500 nm were similarly moved the peak positions by oxidation at the 7^1-position and methylation at the 20-position. The 7^1-oxidation from methyl to the formyl group induces bathochromic shifts of the peaks by 20–30 nm/ca. 800 cm^{-1} and the 20-methylation similarly shifts the peaks by ca. 13 nm/500 cm^{-1}. The bathochromic shift by transformation of 7-CH_3 to 7-CHO is in contrast to the hypsochromic shift of Q_y and Q_y' peaks, while the other bathochromic shift by 20-H to 20-CH_3 is comparable to the similar shift of Q_y and Q_y' peaks. Considering that oxidation of 3-CH_2CH_3 to 3-CHO on the Q_y transition moment of chlorin π-moiety induced the Q_y-peak to shift to ca. a 800 cm^{-1} longer wavelength,[17] and that the 7-position is on the Q_x transition moment, these two bands are assigned as Q_x and Q_x' bands. From comparison with Q_y and Q_y' bands, the peak positioned at the longer wavelength in 500–600 nm is Q_x band and the other at the shorter wavelength is Q_x' band, a vibrational component of Q_x band.

Zinc metallation of all the free base **1**-**4** shifted the Q_y and Soret absorption peaks to shorter and longer wavelengths, respectively, in a diluted dichloromethane solution (see Tables 1 and 2). These shifts are consistent with other reported data.[10,11,14,17,18] The Q_x (and also Q_x') bands in zinc complexes of 7-formyl-chlorins Zn-**2**/**4** were too broad to be detected in the present measurement, however, the observed Q_x and Q_x' peaks of Zn-**1**/**3** were shifted to longer wavelengths by zinc-metallation. Substitution effects on the peak positions in zinc chlorins are similar with those in the free base described above. Exceptions were Q_y and Q_y' peaks which were shifted more hypsochromically by 7-CH_3 in Zn-**1**/**3** to 7-CHO in Zn-**2**/**4** (16±3 nm/420±100 cm^{-1}) compared with those of free base **1**-**4**.

Upon dilution of the dichloromethane solution of zinc chlorins Zn-**1**-**4** by a 99 fold volume of cyclohexane, Q_y and Soret bands were red-shifted and broadened, and the intensity of the Q_y peak became larger than that of the

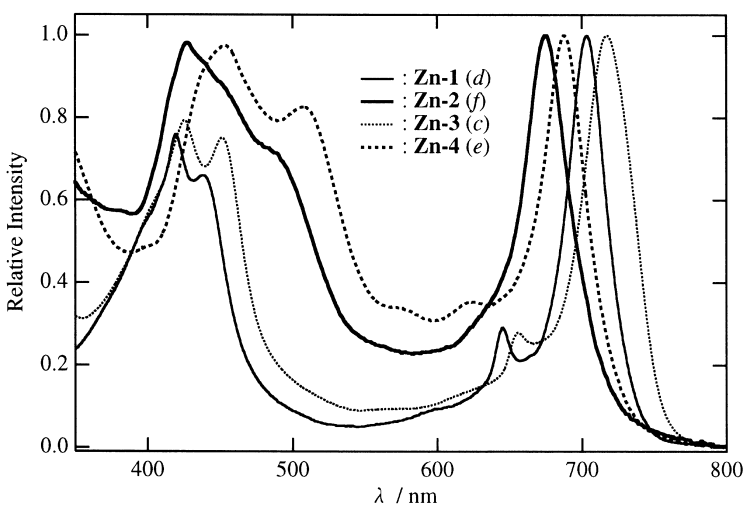


Figure 3. Visible spectra of zinc methyl 8-ethyl-12-methyl-bacteriopheophorbides-*d*/*f*/*c*/*e* (Zn-[E,M]BPhes-*d*/*f*/*c*/e_M, Zn-**1**/**2**/**3**/**4**) in 1% (v/v) CH_2Cl_2-cyclohexane, normalized at each Q_y peak.

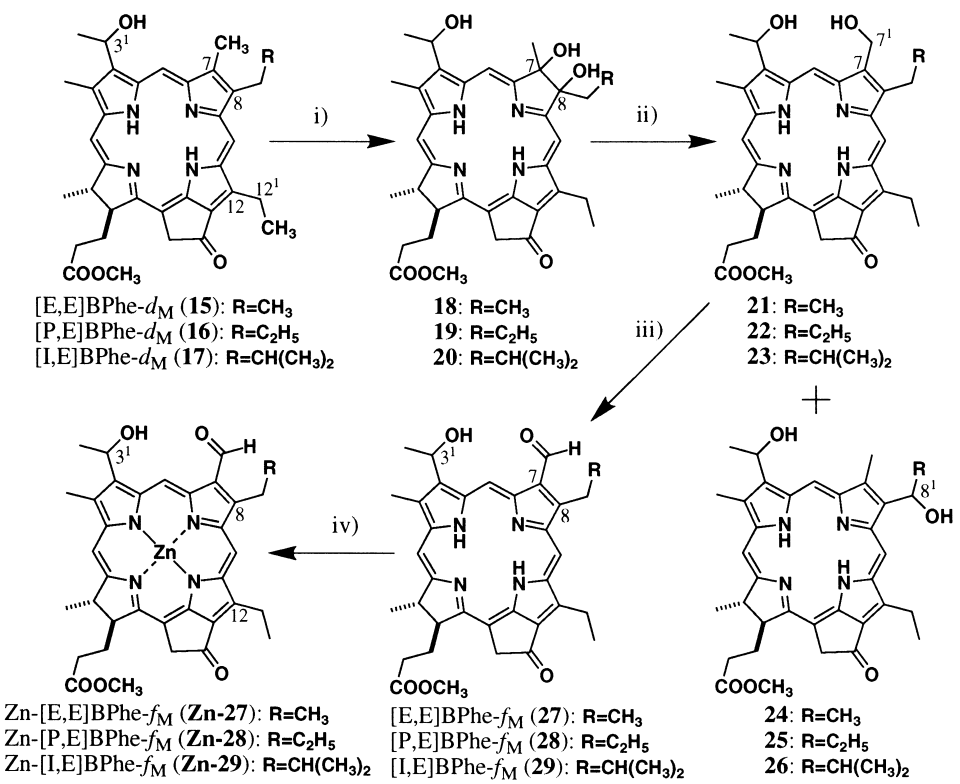
Table 3. Absorption maxima λ_{max} (nm, upper: Q_y and lower: Soret) of Zn-[E,M]BPhe-x_M **1** (*x*=*d*), **2** (*x*=*f*), **3** (*x*=*c*) and **4** (*x*=*e*) monomers in dichloromethane and oligomers in 1% (v/v) dichloromethane and cyclohexane and their red-shifts Δ (cm^{-1}) by self-aggregation

	λ_{max} (monomer)	λ_{max} (oligomer)	Δ
Zn-**1** (R^7=CH_3, R^{20}=H) [$3^1R/S$=1/1]	647	703	1230
	422	438	870
Zn-**2** (R^7=CHO, R^{20}=H) [$3^1R/S$=1/1]	632	675	1010
	450	≈495	≈2000
Zn-**3** (R^7=CH_3, R^{20}=CH_3) [$3^1R/S$=2/1]	658	717	1250
	427	452	1300
Zn-**4** (R^7=CHO, R^{20}=CH_3) [$3^1R/S$=2/1]	643	688	1020
	457	508	2200

Soret peak (Fig. 3). These changes in absorption spectra indicated that Zn-**1**-**4** self-aggregated in the non-polar organic solvent to afford oligomers along the Q_y direction in the supramolecule as reported previously.[10,14,17–19] As listed in Table 3, the red-shifts (Δs) of Q_y peak by self-aggregation of zinc 7-methyl-chlorins Zn-**1**/**3** are almost the same (ca. 1200 cm^{-1}) and those of zinc 7-formyl-chlorins Zn-**2**/**4** are also the same within a reasonable error (ca. 1000 cm^{-1}), indicating that the 20-methyl group did not affect the Δ values in Q_y peak. The former is larger by about 200 cm^{-1} than the latter and the 7^1-oxidation (7-CH_3→7-CHO) suppressed the value. In contrast, the Δ of Soret peak in Zn-**2**/**4** is much larger than Zn-**1**/**3**, respectively, and the 7^1-oxidation increased its value. As a result, self-aggregates of zinc 7-formyl-chlorins Zn-**2**/**4** have relatively large absorption at the region of 500–550 nm, which is usually absorbed by carotenoids. The specific absorption band by such chlorophyllous pigments is consistent with results reported in other 7-formyl-chlorin self-aggregates.[3,14] It is noteworthy that Q_y peaks of the present four self-aggregates are shifted by about a 15-nm interval: Zn-**2**<Zn-**4**<Zn-**1**<Zn-**3** (see Fig. 3 and Table 3).

2.3. Synthesis and visible spectra of methyl bacteriopheophorbide-*e*/*f* homologs

A strain of *Chlorobium vibrioforme* f. sp. *thiosulfatophilum* NCIB 8327 has several BChl-d_F homologs as chlorosomal chlorophylls, and the main ones being 8,12-diethyl ([E,E]), 8-propyl-12-ethyl ([P,E]) and 8-isobutyl-12-ethyl ([I,E]) substituents.[20] All the chlorophylls were extracted from the strain cultured in aqueous media and transformed to methyl pheophorbides by treatment with cold methanol containing sulfuric acid. The resulting BPhes-d_M was purified from the reaction mixture by FCC and the three main homologs **15**-**17** were separated by reverse-phase HPLC. Each separated



Scheme 4. Synthesis of methyl 12-ethyl-bacteriopheophorbide-*f* homologs ([X,E]BPhes-f_M, **27**-**29**) and their zinc complexes (Zn-[X,E]BPhes-f_M, Zn-**27**-**29**) by modification of the corresponding 7-methyl derivatives ([X,E]BPhe-d_M, **15**-**17**); (i) OsO_4–C_5H_5N/CH_2Cl_2, H_2S/MeOH; (ii) aq. HCl/$O(CH_2CH_2)_2O$ at 50°C; (iii) PDC/C_6H_6; (iv) $Zn(OAc)_2{\cdot}2H_2O$/MeOH–CH_2Cl_2.

BPhe-d_M was oxidized by OsO_4 in the presence of pyridine to give the corresponding *cis*-diol **18**-**20** (Scheme 4). The yield from [E,E]-**15** to **18** (57%) was comparable to that from [E,M]-**1** to **5** (56%), indicating there was no remote control for the 7,8-oxidation by 12^1-methylation (from the 12-methyl to 12-ethyl group). Methylation at the 8^2-position decreased the yields to 52% for 8-propyl homolog (**16**→**19**) and 33% for 8-isobutyl (**17**→**20**). A sterically bulky substituent at the 8-position suppressed the 7,8-oxidation. The *cis*-diols were 7,8-diastereomeric mixtures with ratios of about 7–8/6. The *cis*-diols **18**-**20** were mono-dehydrated to give a mixture of 7^1-hydroxy-chlorins **21**-**23** and 8^1-hydroxy-chlorins **24**-**26**, respectively. The mixtures were separated by FCC and **21**-**23** were isolated in 8–9% yields. These values were comparable to that of **7** to [E,M]-**2** (10%), showing that the 8^2-substituents little affected the preparation of 7-hydroxymethyl homologs. Isolated yields of 8^1-hydroxy-homologs **24**-**26** were 71, 66 and 56%, respectively. The gradual decrease of the yield would be ascribable to an increase in the steric hindrance around the 8^1-position. The separated 7-(hydroxymethyl)chlorins **21**-**23** were selectively oxidized to afford 7-formyl homologs, [E,E]-, [P,E]- and [I,E]-BPhes-f_M (**27**-**29**), respectively, in a moderate yield (ca. 50%).

Therefore, several BPhes-d_M homologs **1** and **15** -**17** were converted to the corresponding BPhes-f_M **2** and **27**-**29**, irrespective of any methylation at the 8^2- and 12^1-positions. The stereochemistry at the 3^1-position did not change during the oxidation of the 7-methyl to 7-formyl group; both **15** and **27** were only *R*-epimers, **16** and **28** were 9/1 *R*/*S*-epimeric mixtures and **17** and **29** were only *S*-epimers. The structurally determined BPhe-f_M homologs will be helpful for elucidation of the mechanism in biosynthesis of BChl-*e* (BChlide-*d* (R=H in the left drawing of Fig. 1)→BChlide-*c*→BChlide-*e* or BChlide-*d*→BChlide-*f*→BChlide-*e*)[13] as well as for the first detection of BChl-*f* in chlorophyllous pigments extracted from photosynthetic green bacteria.

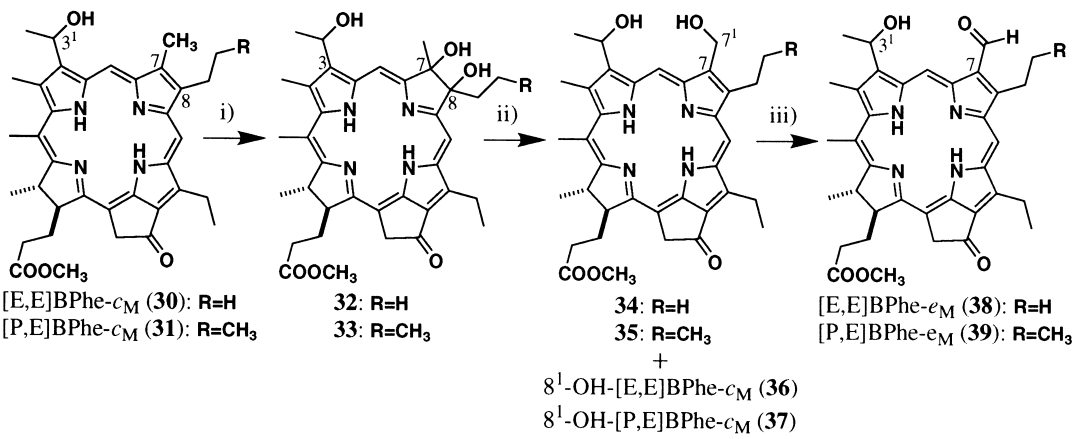
No change was observed in visible spectra of monomeric (Zn-)BPhes-f_M homologs in dichloromethane because any methyl groups at the 8^2- and 12^1-positions did not directly attach to a chlorin π-system. Visible bands of self-aggregates of Zn-BPhes-f_M homologs Zn-**2**/**27**-**29** in 1% (v/v) dichloromethane and cyclohexane were dependent upon the 8- and 12-substituents as well as the 3^1-stereochemistry (see Table 4). These results are similar to the observations in naturally occurring BChl-*c*/*d*/*e*[3,6,21] and synthetic Zn-BPhe-*c* homologs.[19] It is assumed that unknown BChl-*f* homologs and epimers self-aggregated to give oligomers in a manner similar to known BChls-*c*/*d*/*e*.

Table 4. Q_y-absorption maxima λ_{max} (nm) of Zn-BPhes-f_M in 1% (v/v) dichloromethane and cyclohexane and the red-shifts Δ (cm^{-1}) by self-aggregation

	λ_{max} (oligomer)	Δ[a]
Zn-[E,M]-**2** (3^1*R*/*S*=5/5)	675	1010
Zn-[E,E]-**27** (3^1*R*/*S*=10/0)	670	900
Zn-[P,E]-**28** (3^1*R*/*S*=9/1)	677	1050
Zn-[I,E]-**29** (3^1*R*/*S*=0/10)	684	1200

[a] $\Delta=[1/\lambda_{max}$(monomer in dichloromethane: 632 nm)$-1/\lambda_{max}$ (oligomer)]$\times 10^7$.

Synthesis of BPhe-e_M homologs was also examined by similar modification of natural BChls-c_F. A mixture of several BPhe-c_M homologs was prepared from *Chlorobium tepidum* containing various BChls-*c* homologs (vide supra) and separated by HPLC to give [E,E]- and [P,E]-BPhes-c_M **30** and **31** as the main components.[19] The former **30** consisted of only 3^1*R*-epimer and the latter **31** was a 3/1 mixture of 3^1*R*- and 3^1*S*-epimers, which were identical to the epimerical ratios of each naturally occurring BChls-c_F. Using the procedures described above (Scheme 5), OsO_4-oxidation of *R*[E,E]-**30** and *R*/*S*(3/1)[P,E]-**31** gave a 7/6 diastereomeric mixture of *cis*-diols **32** (52%) and **33** (43%; slightly suppressed by a more bulky propyl group at the 8-position), followed by mild mono-dehydration and FCC-separation to afford 7^1-hydroxy-chlorins **34** (12%) and **35** (10%) with concomitant formation of 8^1-hydroxy-chlorins **36** (81%) and **37** (61%). Successively selective PDC-oxidation of the 7-hydroxymethyl group in separated **34** and **35** led to desired *R*[E,E]- and *R*/*S*(3/1)[P,E]-BPhes-e_M **38** (56%) and **39** (52%). All the above transformations were performed without epimerization of the 3^1-position. The 3^1-stereochemistry of any BChls-*e* could be unambiguously determined using a simple modification of structurally determined BChls-*c*[22] to BPhes-e_M with retention of the 3^1-chirality. The present work clearly showed the following HPLC elution pattern: a 3^1*R*-epimer of Zn-BPhe-*c*/*d*/*e*/f_M



Scheme 5. Synthesis of methyl 12-ethyl-bacteriopheophorbide-*e* homologs ([X,E]BPhes-e_M, **38**,**39**) by modification of the corresponding 7-methyl derivatives ([X,E]BPhes-c_M, **30**,**31**); (i) OsO_4–C_5H_5N/CH_2Cl_2, H_2S/MeOH; (ii) aq. $HCl/O(CH_2CH_2)_2O$ at 50°C; (iii) PDC/C_6H_6.

homologs was eluted faster than the corresponding *S*-epimer on a reverse-phase HPLC column (octadecyl type, aqueous methanol, see Section 4). The eluting order observed here and previously[10] is general and is useful for determination of the 3^1-epimerical purity and for assignment of the absolute configuration in natural BChls-*c*/*d*/*e*(/*f*) as well as synthetic (Zn-)BPhe-*c*/*d*/*e*/*f* possessing various hydrocarbon ester groups on the 17-propionate.

3. Conclusion

BChls-*c*/*d* extracted from cultured photosynthetic green bacteria were modified by transformation of the methyl to formyl group at the 7-position to give homologously pure BPhes-*e*/f_M. During the oxidation procedure, no epimerization occurred on the asymmetric 1-hydroxyethyl group at the 3-position. The stereochemistry at the 3^1-position in the synthetic BPhes-*e*/f_M and their zinc complexes (Zn-BPhes-*e*/f_M) was unambiguously determined using the above transformation of structurally known BChls-*c*/*d*. In a reverse-phase HPLC separation, the 3^1*R*-epimer of Zn-BPhe-*c*/*d*/*e*/f_M homologs generally eluted faster than the corresponding *S*-epimer. Visible absorption spectra showed that synthetic (Zn-)BPhes-*c*/*d*/*e*/f_M were monomer in dichloromethane. The oxidation of the 7-methyl to formyl group moved the Soret/Q_x and Q_y peaks to longer and shorter wavelengths, respectively, while all the bands gave bathochromic shifts by the 20-methylation. The monomer peaks were not changed by $8^2/12^1$-methylation (homologs) or 3^1-epimerization (stereoisomers). In 1% (v/v) dichloromethane and cyclohexane, all the synthetic Zn-BPhes-*c*/*d*/*e*/f_M self-aggregated to form large oligomers with a red-shifted Q_y peak, compared to the monomeric. The Q_y peaks in monomeric as well as oligomeric states moved to longer wavelengths in the order of (Zn-)BPhes-*f* (7-CHO/20-H)<*e* (7-CHO/20-CH_3)<*d* (7-CH_3/20-H)<*c* (7-CH_3/20-CH_3).

4. Experimental

4.1. General

All the apparatuses used were described in our previous reports.[20,23] Solvents for visible spectra were purchased from Nacalai Tesque (Grade for UV-spectroscopy). Metal-free chlorins possess two NH at the inner side of the π-conjugate system but, except for **22**, only one NH proton signal was observed because the other NH was too broad to be detected.

4.2. Synthetic procedures

4.2.1. Oxidation of C7–C8 double bond. To 3-(1-hydroxyethyl)chlorin in dry CH_2Cl_2, pyridine and OsO_4 were added and the mixture was stirred at room temperature under N_2. After stirring for several hours, the reaction mixture was diluted with MeOH and bubbled with H_2S for 25 min.[7,11] The mixture was filtered and evaporated under reduced pressure. The residue was purified by FCC (eluted with 3% MeOH–CH_2Cl_2) and recrystallized from CH_2Cl_2–hexane to give a 7,8-*cis*-diol. The 7,8-*cis*-diol was a mixture of 7*R*,8*S*- and 7*S*,8*R*-stereoisomers.

4.2.2. Dehydration of 7,8-*cis*-diol. To the above 7,8-*cis*-diol in 1,4-dioxane, conc. HCl and distilled H_2O were added and the solution was stirred at 50°C under N_2.[7,8] After stirring for a few minutes, the reaction mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with aq. $NaHCO_3$ (4%) and water, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified and separated by FCC (eluted with 1.0–1.5% MeOH–CH_2Cl_2), then recrystallized from CH_2Cl_2–hexane to give 8-(1-hydroxyethyl)chlorin (first fraction) and 7-(hydroxymethyl)chlorin (second fraction). The former secondary alcohol was an 8^1-epimeric mixture (1/1).

4.2.3. Oxidation of 7-hydroxymethyl group. To the above 7-(hydroxymethyl)chlorin (10 μmol) in benzene (8 ml), PDC (5.6 mg, 15 μmol) was added and stirred for 4 h at room temperature under N_2.[14] After addition of Et_2O, the reaction mixture was stirred for a short time, washed with aq. NaCl, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by HPLC (HITACHI GL-OP100 with MeOH, 1.5 ml/min) and recrystallized from CH_2Cl_2–hexane to give 7-formyl-chlorin.

4.2.4. Zinc-metallation of metal-free 7-formyl-chlorin. According to the reported procedures,[18] a reaction of the above 7-formyl-chlorin in CH_2Cl_2 with saturated zinc acetate dihydrate in MeOH for a few hours at room temperature under N_2 with stirring gave the corresponding zinc chlorin after purification by HPLC (HITACHI GL-OP100 with MeOH only, 1.5 ml/min). The resulting zinc complex was precipitated from hexane to give light brown solid as a pure sample; mp>300°C. The analytical separation of the 3^1*R*/*S*-epimers was performed by HPLC (Cosmosil 5C18-ARII 6 mmϕ×250 mm, H_2O–MeOH, 1.5 ml/min).

4.2.5. Preparation of methyl bacteriopheophorbide homologs. After complete cultivation of a green sulfur bacterium, the cultured solution (1 l) was centrifuged to give a green species by decantation. MeOH (20 ml) was added to the green substance (ca. 3 g) and the suspension was vigorously stirred for 30 min under N_2. After filtration, the residue was repeatedly extracted with MeOH–petroleum ether (1/1, 100 ml) until the filtrate was colorless (three times at least). To each filtrate, 1% (v/v) conc. HCl–brine (10 ml) was added with stirring and the organic phase was separated. The aqueous phase was re-extracted with several portion of diethyl ether–petroleum ether (1/1), and the combined organic phases were washed with H_2O and dried over Na_2SO_4. After evaporation, the residue was suspended in MeOH (10 ml), to which was added ice-chilled 20% (v/v) conc. H_2SO_4–MeOH (80 ml) at 0°C under N_2. After 1 h-stirring, the solution was poured into ice-water, extracted with CH_2Cl_2, washed with H_2O several times, dried over Na_2SO_4 and evaporated to dryness. The residue was purified with FCC (eluted with 1% MeOH–CH_2Cl_2) to give a mixture of homologs. Homologs were separated by HPLC (Cosmosil 5C18-ARII 6 mmϕ×250 mm, 10% H_2O–MeOH, 1.5 ml/min) and recrystallization from

CH_2Cl_2–hexane to afford pure samples. On the reverse-phase HPLC, the homologs were eluted in the order of molecular hydrophobicity, consistent with the methylation number at the 8^2-position.[20]

4.3. Synthetic compounds

4.3.1. 7,8-*cis*-Dihydroxy adduct of [E,M]BPhe-d_M (5). Oxidation of [E,M]BPhe-d_M (**1** prepared by modification of Chl-*a* from commercially available *Spirulina geitleri*,[10] 73.8 mg, 0.13 mmol) with pyridine (0.595 ml) and OsO_4 (50.0 mg, 0.16 mmol) in dry CH_2Cl_2 (16.0 ml) for 10 h gave 7,8-*cis*-diol **5** (41.3 mg, 56% yield, $3^1R/3^1S$=1/1, a 7/6 mixture of 7,8-stereoisomers); vis (CH_2Cl_2) λ_{max}=707 (relative intensity, 68), 646 (30), 518 (67), 486 (23), 454 (13), 357 (100) nm; ^{1}H NMR ($CDCl_3$) δ=8.74/8.66/8.65/8.63 (1H, s, 5-H, 7/6/7/6), 8.42/8.41/8.39/8.38 (1H, s, 10-H), 8.05/8.04/8.03/8.02 (1H, s, 20-H), 6.17–6.22 (1H, m, 3-CH), 4.88, 4.70 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.00–4.15 (1H, m, 18-H), 3.63–3.97 (1H, m, 17-H), 3.64/3.61 (3H, s, $COOCH_3$), 3.30 (3H, s, 12-CH_3), 3.23/3.20 (3H, s, 2-CH_3), 2.10–2.75 (6H, m, 8-CH_2, 17-CH_2CH_2), 2.04/2.03 (3H, d, J=7 Hz, 3^1-CH_3), 1.95/1.92/1.86/1.85 (3H, s, 7-CH_3), 1.77/1.77/1.68/1.65 (3H, d, J=7 Hz, 18^1-CH_3), 1.28/1.25/1.15/1.13 (3H, t, J=7 Hz, 8^1-CH_3), −0.15/−0.18/−0.30 (1H, s, NH). MS (FAB) found: m/z 600. Calcd for $C_{34}H_{40}N_4O_6$: M^+, 600.

4.3.2. 7^1-Hydroxy-[E,M]BPhe-d_M (7) and 8^1-hydroxy-[E,M]BPhe-d_M (8). Dehydration of 7,8-*cis*-diol **5** (9.4 mg, 15 μmol) with conc. HCl (120 μl) and H_2O (1.4 ml) in 1,4-dioxane (7 ml) for 5 min gave **7**[14] (0.9 mg, 10% yield, $3^1R/3^1S$=1/1, see spectral data in Ref. 14) and **8** (7.3 mg, 81% yield, $3^1R/3^1S$=1/1, $8^1R/8^1S$=1/1).

Compound **8**. Vis (CH_2Cl_2) λ_{max}=658 (rel. 43), 603 (11), 538 (9), 507 (9), 412 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.94, 9.76/9.73/9.72/9.67 (1/1/1/1), 8.46/8.45 (each 1H, s, 5-, 10-, 20-H), 6.37–6.44 (1H, m, 3-CH), 6.20–6.22 (1H, m, 8-CH), 4.87–5.11 (2H, m, 13^1-CH_2), 4.36–4.40 (1H, m, 18-H), 4.10–4.12 (1H, m, 17-H), 3.64, 3.62/3.61, 3.45/3.43, 3.33 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.47–2.54, 2.18–2.24 (each 2H, m, 17-CH_2CH_2), 2.13 (3H, d, J=7 Hz, 3^1-CH_3) 2.08 (3H, d, J=7 Hz, 18-CH_3), 1.83 (3H, m, 8^1-CH_3), −2.04 (1H, s, NH). MS (FAB) found: m/z 583. Calcd for $C_{34}H_{38}N_4O_5$: M^+, 583.

4.3.3. Methyl 8-ethyl-12-methyl-bacteriopheophorbide-*f* ([E,M]BPhe-f_M, 2). Oxidation of 7-(hydroxymethyl)-chlorin **7** (6.2 mg, 11 μmol) with PDC gave **2**[14] (4.9 mg, 79% yield, $3^1R/3^1S$=1/1, retention time (rt)=18 min); see spectral data in Ref. 14.

4.3.4. 7,8-*cis*-Dihydroxy adduct of [E,M]BPhe-c_M (12). Oxidation of [E,M]BPhe-c_M (**3** from cultured *Chloroflexus aurantiacus*[10] 27.0 mg, 0.047 mmol) with pyridine (0.446 ml) and OsO_4 (57 mg, 0.18 mmol) in dry CH_2Cl_2 (6 ml) for 4.5 h gave 7,8-*cis*-diol **12** (17.5 mg, 67% yield, $3^1R/3^1S$=2/1, a 7/6 mixture of 7,8-stereoisomers); vis (CH_2Cl_2) λ_{max}=719 (rel. 36), 655 (13), 534 (34), 502 (12), 370 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.02/8.85/8.78/8.75, 8.43/8.42/8.39/8.37 (each 1H, s, 5-, 10-H), 6.24–6.40 (1H, m, 3-CH), 4.85/4.84 (2H, s, 13^1-CH_2), 4.10–4.25 (1H, m, 18-H), 3.83–3.97 (1H, m, 17-H), 3.64/3.60, 3.59/3.56, 3.40/3.35, 3.23/3.19 (each 3H, s, 2-, 12-, 20-CH_3, $COOCH_3$), 2.29–2.55 (6H, m, 8-CH_2, 17-CH_2CH_2), 2.05/2.04 (3H, d, J=7 Hz, 3^1-CH_3), 1.96/1.88 (3H, s, 7-CH_3), 1.38/1.29/1.25/1.19 (3H, d, J=7 Hz, 18^1-CH_3), 1.06/0.96 (3H, t, J=7 Hz, 8-CH_3), −0.17/−0.90/−0.30 (1H, s, NH). MS (FAB) found: m/z 614. Calcd for $C_{35}H_{42}N_4O_6$: M^+, 614.

4.3.5. 7^1-Hydroxy-[E,M]BPhe-c_M (13) and 8^1-hydroxy-[E,M]BPhe-c_M (14). Dehydration of 7,8-*cis*-diol **12** (15.9 mg, 26 μmol) with conc. HCl (300 μl) and H_2O (2.3 ml) in 1,4-dioxane (12 ml) for 1 min gave 7-(hydroxymethyl)chlorin **13** (1.6 mg, 10% yield, $3^1R/3^1S$=2/1) and 8-(1-hydroxyethyl)chlorin **14** (12.8 mg, 80% yield, $3^1R/3^1S$=2/1, $8^1R/8^1S$=1/1).

Compound **13**. Vis (CH_2Cl_2) λ_{max}=656 (rel. 41), 610 (9), 555 (9), 520 (11), 419 (100) nm; ^{1}H NMR ($CDCl_3$) δ ($3^1R/3^1S$=2/1)=10.01/10.10, 9.41/9.43 (each 1H, s, 5-, 10-H), 6.54 (1H, q, J=7 Hz, 3-CH), 5.78 (2H, s, 7-CH_2), 5.18 (2H, s, 13^1-CH_2), 4.53–4.60 (1H, m, 18-H), 4.14–4.17 (1H, m, 17-H), 3.88 (3H, s, 20-CH_3), 3.78 (2H, q, J=7 Hz, 8-CH_2), 3.61/3.60, 3.59, 3.50/3.49 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.42–2.52, 2.34–2.40 (each 2H, m, 17-CH_2CH_2), 2.15 (3H, d, J=7 Hz, 3^1-CH_3), 1.73 (3H, t, J=7 Hz, 8^1-CH_3), 1.48 (3H, d, J=7 Hz, 18-CH_3), −1.88 (1H, s, NH). MS (FAB) found: m/z 596. Calcd for $C_{35}H_{40}N_4O_5$: M^+, 596.

Compound **14**. Vis (CH_2Cl_2) λ_{max}=658 (rel. 43), 608 (11), 553 (9), 520 (9), 417 (100) nm; ^{1}H NMR ($CDCl_3$) δ=10.0/9.99/9.97/9.94, 9.88/9.86 (each 1H, s, 5-, 10-H), 6.45–6.53 (1H, m, 3-CH), 6.17–6.20 (1H, m, 8-CH), 5.14 (2H, s, 13^1-CH_2), 4.54–4.58 (1H, m, 18-H), 4.11–4.18 (1H, m, 17-H), 3.88 (3H, s, 20-CH_3), 3.62/3.61, 3.59/3.57, 3.52/3.51, 3.37/3.36 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.65–2.89, 2.41–2.59 (each 2H, m, 17-CH_2CH_2), 2.14/2.12 (3H, d, J=7 Hz, 3^1-CH_3), 1.45 (3H, m, 8^1-CH_3), 1.33 (3H, d, J=7 Hz, 18-CH_3), −2.09 (1H, s, NH). MS (FAB) found: m/z 596. Calcd for $C_{35}H_{40}N_4O_5$: M^+, 596.

4.3.6. Methyl 8-ethyl-12-methyl-bacteriopheophorbide-*e* ([E,M]BPhe-e_M, 4). Oxidation of 7-(hydroxymethyl)-chlorin **13** (1.3 mg, 2 μmol) with PDC gave **4** (0.7 mg, 52% yield, $3^1R/3^1S$=2/1, rt=20 min); vis (CH_2Cl_2) λ_{max}=660 (rel. 18), 605 (6), 575 (6), 537 (8), 445 (100) nm; ^{1}H NMR ($CDCl_3$) δ ($3^1R/3^1S$=2/1)=11.25 (1H, s, CHO), 10.76, 9.66 (each 1H, s, 5-, 10-H), 6.66 (1H, q, J=7 Hz, 3-CH), 5.22 (2H, s, 13^1-CH_2), 4.60 (1H, dq, J=2, 7 Hz, 18-H), 4.26 (1H, m, 17-H), 4.11/4.06 (2H, q, J=8 Hz, 8-CH_2), 3.89 (3H, s, 20-CH_3), 3.69, 3.59/3.57, 3.44 (each 3H, s, 2-, 12-CH_3, $COOCH_3$), 2.50–2.65, 2.24–2.30 (each 2H, m, 17-CH_2CH_2), 2.20 (3H, d, J=7 Hz, 3^1-CH_3) 1.85 (3H, t, J=8 Hz, 8^1-CH_3), 1.34 (3H, d, J=7 Hz, 18-CH_3), −1.60 (1H, s, NH). MS (FAB) found: m/z 594. Calcd for $C_{35}H_{38}N_4O_5$: M^+, 594.

4.3.7. Methyl bacteriopheophorbides-*d*; [E,E]BPhe-d_M (15), [P,E]BPhe-d_M (16) and [I,E]BPhe-d_M (17). A mixture of BPhe-d_M homologs from a strain of *Chlorobium vibrioforme*[5,20] was separated by HPLC to give three homologously pure samples as follows. [E,E]-**15** (3^1R

only), [P,E]-**16** ($3^1R/3^1S$=9/1) and [I,E]-**17** (3^1S only) were eluted in the order.[24]

Compound **15**.[24] Vis (CH_2Cl_2) λ_{max}=660 (rel. 46), 603 (7), 535 (9), 505 (9), 409 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.58, 9.48, 8.48 (each 1H, s, 5-, 10-, 20-H), 6.28 (1H, q, J=7 Hz, 3-CH), 5.28, 5.11 (2H, d, J=20 Hz, 13^1-CH_2), 4.42–4.45 (1H, m, 18-H), 4.22–4.26 (1H, m, 17-H), 4.06 (2H, q, J=8 Hz, 12-CH_2), 3.67 (2H, q, J=7 Hz, 8-CH_2), 3.62, 3.35, 3.22 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.48–2.63, 2.18–2.29 (each 2H, m, 17-CH_2CH_2), 2.09 (3H, d, J=7 Hz, 3^1-CH_3), 1.93 (3H, t, J=8 Hz, 12^1-CH_3), 1.77 (3H, d, J=7 Hz, 18-CH_3), 1.68 (3H, t, J=7 Hz, 8^1-CH_3), −1.79 (1H, s, NH). MS[25] (FAB) found: m/z 580. Calcd for $C_{35}H_{40}N_4O_4$: M^+, 580.

Compound **16**.[24] Vis (CH_2Cl_2) λ_{max}=660 (rel. 46), 603 (7), 535 (9), 505 (9), 409 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.59/9.56, 9.43, 8.47/8.46 (each 1H, s, 5-,10-, 20-H), 6.28 (1H, q, J=7 Hz, 3-CH), 5.20, 5.08 (2H, d, J=20 Hz, 13^1-CH_2), 4.41–4.44 (1H, m, 18-H), 4.20–4.23 (1H, m, 17-H), 4.04 (2H, q, J=7 Hz, 12-CH_2), 3.56 (2H, t, J=7 Hz, 8-CH_2), 3.64/3.63, 3.35, 3.20 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.51–2.60, 2.18–2.26 (each 2H, m, 17-CH_2CH_2), 2.09–2.16 (2H, m, 8^1-CH_2), 2.08 (3H, d, J=7 Hz, 3^1-CH_3), 1.92 (3H, t, J=7 Hz, 12^1-CH_3), 1.76 (3H, d, J=7 Hz, 18-CH_3), 1.22 (3H, t, J=7 Hz, 8^2-CH_3), −1.82 (1H, s, NH). MS[25] (FAB) found: m/z 594. Calcd for $C_{36}H_{42}N_4O_4$: M^+, 594.

Compound **17**.[24] Vis (CH_2Cl_2) λ_{max}=660 (rel. 46), 603 (7), 535 (9), 505 (9), 409 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.67, 9.48, 8.49 (each 1H, s, 5-,10-, 20-H), 6.38 (1H, q, J=7 Hz, 3-CH), 5.23, 5.08 (2H, d, J=20 Hz, 13^1-CH_2), 4.43–4.46 (1H, m, 18-H), 4.23–4.26 (1H, m, 17-H), 4.08 (2H, q, J=7 Hz, 12-CH_2), 3.54 (2H, d, J=7 Hz, 8-CH_2), 3.62, 3.38, 3.24 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.51–2.67, 2.18–2.30 (each 2H, m, 17-CH_2CH_2), 2.44–2.49 (1H, m, 8^1-CH), 2.13 (3H, d, J=7 Hz, 3^1-CH_3), 1.92 (3H, t, J=7 Hz, 12^1-CH_3), 1.76 (3H, d, J=7 Hz, 18-CH_3), 1.23 (6H, d, J=7 Hz, 8^2-CH_3), −1.77 (1H, s, NH). MS[25] (FAB) found: m/z 608. Calcd for $C_{37}H_{44}N_4O_4$: M^+, 608.

4.3.8. 7,8-*cis*-Dihydroxy adduct of [E,E]BPhe-d_M (18). Oxidation of [E,E]BPhe-d_M (**15**, 58.9 mg, 0.10 mmol) with pyridine (0.95 ml) and OsO_4 (128 mg, 0.4 mmol) in dry CH_2Cl_2 (12 ml) for 3.5 h gave 7,8-*cis*-diol **18** (34.4 mg, 57% yield, 3^1R only, a 7/6 mixture of 7,8-stereoisomers); vis (CH_2Cl_2) λ_{max}=707 (rel. 68), 646 (30), 518 (67), 486 (23), 454 (13), 357 (100) nm; ^{1}H NMR ($CDCl_3$) δ=8.65/8.63, 8.45/8.43, 8.04/8.02 (each 1H, s, 5-, 10-, 20-H), 6.19–6.24 (1H, m, 3-CH), 4.92, 4.85 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.11–4.15 (1H, m, 18-H), 3.95–3.98 (1H, m, 17-H), 3.70–3.73 (2H, m, 12-CH_2), 3.63/3.62, 3.23/3.22, (each 3H, s, 2-CH_3, $COOCH_3$), 2.42–2.60 (6H, m, 8-CH_2, 17-CH_2CH_2), 2.03 (3H, d, J=7 Hz, 3^1-CH_3), 1.92/1.86 (3H, s, 7-CH_3), 1.78/1.75 (3H, t, J=7 Hz, 12^1-CH_3), 1.67/1.63 (3H, d, J=7 Hz, 18^1-CH_3), 1.26/1.14 (3H, t, J=8 Hz, 8^1-CH_3), −0.23/−0.11 (1H, s, NH). MS (FAB) found: m/z 614. Calcd for $C_{35}H_{42}N_4O_6$: M^+, 614.

4.3.9. 7,8-*cis*-Dihydroxy adduct of [P,E]BPhe-d_M (19). Oxidation of [P,E]BPhe-d_M (**16**, 60.0 mg, 0.10 mmol) with pyridine (0.99 ml) and OsO_4 (128 mg, 0.4 mmol) in dry CH_2Cl_2 (12.1 ml) for 4.5 h gave 7,8-*cis*-diol **19** (31.6 mg, 52% yield, $3^1R/3^1S$=9/1, a 7/6 mixture of 7,8-stereoisomers); vis (CH_2Cl_2) λ_{max}=707 (rel. 68), 646 (30), 518 (67), 486 (23), 454 (13), 357 (100) nm; ^{1}H NMR ($CDCl_3$) δ=8.65/8.62, 8.46/8.44, 8.04/8.02 (each 1H, s, 5-, 10-, 20-H), 6.18–6.24 (1H, m, 3-CH), 4.92, 4.75 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.18–4.22 (1H, m, 18-H), 4.03–4.06 (1H, m, 17-H), 3.77 (2H, q, J=7 Hz, 12-CH_2), 3.63/3.62, 3.16/3.13 (each 3H, s, 2-CH_3, $COOCH_3$), 2.05–2.36 (6H, m, 8-CH_2, 17-CH_2CH_2), 2.03 (3H, d, J=7 Hz, 3^1-CH_3), 1.91/1.84 (3H, s, 7-CH_3), 1.72–1.77 (2H, m, 8^1-CH_2), 1.66 (3H, t, J=7 Hz, 12^1-CH_3), 1.42/1.41 (3H, d, J=7 Hz, 18^1-CH_3), 1.06/1.10 (3H, t, J=8 Hz, 8^2-CH_3), −0.12/−0.21 (1H, s, NH). MS (FAB) found: m/z 628. Calcd for $C_{36}H_{44}N_4O_6$: M^+, 628.

4.3.10. 7,8-*cis*-Dihydroxy adduct of [I,E]BPhe-d_M (20). Oxidation of [I,E]BPhe-d_M (**17**, 43.2 mg, 0.07 mmol) with pyridine (0.72 ml) and OsO_4 (90 mg, 0.28 mmol) in dry CH_2Cl_2 (8.6 ml) for 6 h gave 7,8-*cis*-diol **20** (15.5 mg, 33% yield, 3^1S only, a 4/3 mixture of 7,8-stereoisomers); vis (CH_2Cl_2) λ_{max}=707 (rel. 68), 646 (30), 518 (67), 486 (23), 454 (13), 357 (100) nm; ^{1}H NMR ($CDCl_3$) δ=8.75/8.59, 8.44/8.37, 7.99/7.93 (each 1H, s, 5-, 10-, 20-H), 6.12–6.20 (1H, m, 3-CH), 4.98, 4.81 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.23–4.28 (1H, m, 18-H), 3.95–4.06 (1H, m, 17-H), 3.69 (2H, q, J=7 Hz, 12-CH_2), 3.63/3.61, 3.19/3.12 (each 3H, s, 2-CH_3, $COOCH_3$), 2.10–2.41 (6H, m, 8-CH_2, 17-CH_2CH_2), 2.04 (3H, d, J=7 Hz, 3^1-CH_3), 1.90/1.83 (3H, s, 7-CH_3), 1.76–1.84 (1H, m, 8^1-CH), 1.64 (3H, t, J=7 Hz, 12^1-CH_3), 1.42/1.41 (3H, d, J=7 Hz, 18^1-CH_3), 1.05 (6H, m, 8^2-CH_3), −0.09/−0.26 (1H, s, NH). MS (FAB) found: m/z 642. Calcd for $C_{37}H_{46}N_4O_6$: M^+, 642.

4.3.11. 7^1-Hydroxy-[E,E]BPhe-d_M (21) and 8^1-hydroxy-[E,E]BPhe-d_M (24). Dehydration of 7,8-*cis*-diol **18** (36.0 mg, 59 μmol) with conc. HCl (642 μl) and H_2O (5.5 ml) in 1,4-dioxane (26.3 ml) for 1 min gave 7-(hydroxymethyl)chlorin **21** (3.0 mg, 9% yield, 3^1R only) and 8-(1-hydroxyethyl)chlorin **24** (24.3 mg, 71% yield, 3^1R only, $8^1R/8^1S$=1/1).

Compound **21**. Vis (CH_2Cl_2) λ_{max}=656 (rel. 41), 609 (9), 539 (9), 507 (11), 414 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.84, 9.59, 8.51 (each 1H, s, 5-, 10-, 20-H), 6.43 (1H, q, J=7 Hz, 3-CH), 5.75 (2H, s, 7-CH_2), 5.24, 5.09 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.44–4.47 (1H, m, 18-H), 4.27–4.32 (1H, m, 17-H), 4.07 (2H, q, J=8 Hz, 12-CH_2), 3.79 (2H, q, J=7 Hz, 8-CH_2), 3.61, 3.49 (each 3H, s, 2-CH_3, $COOCH_3$), 2.52–2.65, 2.23–2.34 (each 2H, m, 17-CH_2CH_2), 2.13 (3H, d, J=7 Hz, 3^1-CH_3), 1.93 (2H, t, J=8 Hz, 12^1-CH_3), 1.79 (3H, d, J=7 Hz, 18-CH_3), 1.74 (3H, t, J=7 Hz, 8^1-CH_3), −1.77 (1H, s, NH). MS (FAB) found: m/z 596. Calcd for $C_{35}H_{40}N_4O_5$: M^+, 596.

Compound **24**. Vis (CH_2Cl_2) λ_{max}=658 (rel. 43), 603 (11), 538 (9), 507 (9), 412 (100) nm; ^{1}H NMR ($CDCl_3$) δ (1/1)=9.91/9.90, 9.59/9.52, 8.40/8.38 (each 1H, s, 5-, 10-, 20-H), 6.24–6.34 (1H, m, 3-CH), 6.11–6.18 (1H, m, 8-CH), 4.60–4.93 (2H, m, 13^1-CH_2), 4.26–4.29 (1H, m, 18-H), 4.00–4.03 (1H, m, 17-H), 3.98 (2H, q, J=8 Hz, 12-CH_2), 3.64/3.63, 3.35/3.32, 3.24 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.38–2.45, 2.12–2.17 (each 2H, m,

17-CH_2CH_2), 2.05 (6H, d, J=7 Hz, 3^1-, 8^1-CH_3), 1.83 (3H, t, J=8 Hz, 12^1-CH_3), 1.64/1.63 (3H, d, J=7 Hz, 18-CH_3), −1.89/−1.93 (1H, s, NH). MS (FAB) found: m/z 596. Calcd for $C_{35}H_{40}N_4O_5$: M^+, 596.

4.3.12. 7^1-Hydroxy-[P,E]BPhe-d_M (22) and 8^1-hydroxy-[P,E]BPhe-d_M (25). Dehydration of 7,8-*cis*-diol **19** (30.0 mg, 47 μmol) with conc. HCl (534 μl) and H_2O (4.6 ml) in 1,4-dioxane (21.8 ml) for 2 min gave 7-(hydroxymethyl)chlorin **22** (2.5 mg, 8% yield, 3^1R/3^1S=9/1) and 8-(1-hydroxyethyl)chlorin **25** (20.1 mg, 66% yield, 3^1R/3^1S=9/1, 8^1R/8^1S=1/1).

Compound **22**. Vis (CH_2Cl_2) λ_{max}=656 (rel. 41), 601 (9), 539 (9), 507 (11), 414 (100) nm; ^{1}H NMR ($CDCl_3$) δ (3^1R/3^1S=9/1)=9.86/9.88, 9.56, 8.51/8.50 (each 1H, s, 5-, 10-, 20-H), 6.42 (1H, q, J=7 Hz, 3-CH), 5.74 (2H, s, 7-CH_2), 5.24, 5.08 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.45–4.49 (1H, m, 18-H), 4.24–4.28 (1H, m, 17-H), 4.06 (2H, q, J=8 Hz, 12-CH_2), 3.73 (2H, t, J=8 Hz, 8-CH_2), 3.61, 3.40/3.38 (each 3H, s, 2-CH_3, $COOCH_3$), 2.52–2.67, 2.23–2.30 (each 2H, m, 17-CH_2CH_2), 2.13 (3H, d, J=7 Hz, 3^1-CH_3), 1.93 (2H, t, J=8 Hz, 12^1-CH_3), 1.79 (3H, d, J=7 Hz, 18-CH_3), 1.60–1.63 (3H, m, 8^1-CH_2), 1.22 (3H, t, J=7 Hz, 8^2-CH_3), 0.33, −1.76 (each 1H, s, NH). MS (FAB) found: m/z 610. Calcd for $C_{36}H_{42}N_4O_5$: M^+, 610.

Compound **25**. Vis (CH_2Cl_2) λ_{max}=658 (rel. 43), 603 (11), 538 (9), 507 (9), 412 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.93/9.92, 9.64/9.63/9.56, 8.41 (each 1H, s, 5-, 10-, 20-H), 6.29–6.38 (1H, m, 3-CH), 5.83–5.88 (1H, m, 8-CH), 4.50–4.70 (2H, m, 13^1-CH_2), 4.29–4.32 (1H, m, 18-H), 4.02–4.05 (1H, m, 17-H), 4.01 (2H, q, J=8 Hz, 12-CH_2), 3.64/3.63, 3.35/3.34/3.32, 3.26 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.16–2.56 (6H, m, 8^1-CH_2, 17-CH_2CH_2), 2.09/2.08 (3H, d, J=7 Hz, 3^1-CH_3) 1.85 (3H, t, J=8 Hz, 12^1-CH_3), 1.68/1.65 (3H, d, J=7 Hz, 18-CH_3), 1.09/1.08 (3H, t, J=7 Hz, 8^2-CH_3), −2.04/−2.06/−2.09 (1H, s, NH). MS (FAB) found: m/z 610. Calcd for $C_{36}H_{42}N_4O_5$: M^+, 610.

4.3.13. 7^1-Hydroxy-[I,E]BPhe-d_M (23) and 8^1-hydroxy-[I,E]BPhe-d_M (26). Dehydration of 7,8-*cis*-diol **20** (15.5 mg, 25 μmol) with conc. HCl (320 μl) and H_2O (2.7 ml) in 1,4-dioxane (13.1 ml) for 2 min gave 7-(hydroxymethyl)chlorin **23** (1.5 mg, 9% yield, 3^1S only) and 8-(1-hydroxyethyl)chlorin **26** (9.1 mg, 56% yield, 3^1S only, 8^1R/8^1S=1/1).

Compound **23**. Vis (CH_2Cl_2) λ_{max}=656 (rel. 41), 601 (9), 539 (9), 507 (11), 414 (100) nm; ^{1}H NMR ($CDCl_3$) δ=9.89, 9.49, 8.47 (each 1H, s, 5-, 10-, 20-H), 6.35 (1H, q, J=7 Hz, 3-CH), 5.70 (2H, s, 7-CH_2), 5.20, 5.06 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.43–4.40 (1H, m, 18-H), 4.21–4.24 (1H, m, 17-H), 4.03 (2H, q, J=8 Hz, 12-CH_2), 3.61 (2H, d, J=8 Hz, 8-CH_2), 3.62, 3.32 (each 3H, s, 2-CH_3, $COOCH_3$), 2.43–2.59, 2.10–2.17 (each 2H, m, 17-CH_2CH_2), 2.11 (3H, d, J=7 Hz, 3^1-CH_3), 1.91 (2H, t, J=8 Hz, 12^1-CH_3), 1.76 (3H, d, J=7 Hz, 18-CH_3), 1.60–1.63 (1H, m, 8^1-CH), 1.20 (6H, t, J=7 Hz, 8^2-CH_3), −1.78 (1H, s, NH). MS (FAB) found: m/z 624. Calcd for $C_{37}H_{44}N_4O_5$: M^+, 624.

Compound **26**. Vis (CH_2Cl_2) λ_{max}=658 (rel. 43), 603 (11), 538 (9), 507 (9), 412 (100) nm; ^{1}H NMR ($CDCl_3$) δ (1/1)=10.01/10.00, 9.74/9.73, 8.51/8.48 (each 1H, s, 5-, 10-, 20-H), 6.37–6.45 (1H, m, 3-CH), 5.57 (1H, d, J=7 Hz, 8-CH), 5.00–5.22 (2H, m, 13^1-CH_2), 4.42–4.45 (1H, m, 18-H), 4.12–4.15 (1H, m, 17-H), 4.07 (2H, q, J=8 Hz, 12-CH_2), 3.64/3.63, 3.40/3.39, 3.33/3.32 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.20–2.74 (5H, m, 8^1-CH, 17-CH_2CH_2), 2.14 (3H, d, J=7 Hz, 3^1-CH_3) 1.92/1.90 (3H, t, J=8 Hz, 12^1-CH_3), 1.76/1.72 (3H, d, J=7 Hz, 18-CH_3), 1.02/1.01 (6H, d, J=8 Hz, 8^2-CH_3), −1.88/−1.95 (1H, s, NH). MS (FAB) found: m/z 624. Calcd for $C_{37}H_{44}N_4O_5$: M^+, 624.

4.3.14. Methyl 8,12-diethyl-bacteriopheophorbide-*f* ([E,E]BPhe-f_M, 27). Oxidation of 7-(hydroxymethyl)-chlorin **21** (3.0 mg, 5 μmol) with PDC gave **27** (1.6 mg, 54% yield, 3^1R only, rt=19 min); vis (CH_2Cl_2) λ_{max}=649 (rel. 21), 595 (6), 562 (6), 525 (7), 435 (100) nm; ^{1}H NMR ($CDCl_3$) δ=11.16 (1H, s, CHO), 10.47, 9.65, 8.51 (each 1H, s, 5-, 10-, 20-H), 6.53 (1H, q, J=7 Hz, 3-CH), 5.23, 5.10 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.46–4.49 (1H, m, 18-H), 4.26–4.29 (1H, m, 17-H), 4.03–4.11 (4H, m, 8-, 12-CH_2), 3.64, 3.45 (each 3H, s, 2-CH_3, $COOCH_3$), 2.53–2.64, 2.27–2.34 (each 2H, m, 17-CH_2CH_2), 2.16 (3H, d, J=7 Hz, 3^1-CH_3) 1.94 (3H, t, J=7 Hz, 12^1-CH_3), 1.76–1.83 (6H, m, 8^1-, 18-CH_3), −1.56 (1H, s, NH). MS (FAB) found: m/z 594. Calcd for $C_{35}H_{38}N_4O_5$: M^+, 594.

4.3.15. Methyl 8-propyl-12-ethyl-bacteriopheophorbide-*f* ([P,E]BPhe-f_M, 28). Oxidation of 7-(hydroxymethyl)chlorin **22** (2.5 mg, 4 μmol) with PDC gave **28** (1.2 mg, 49% yield, 3^1R/3^1S=9/1, rt=19 min); vis (CH_2Cl_2) λ_{max}=649 (rel. 21), 595 (6), 562 (6), 525 (7), 435 (100) nm; ^{1}H NMR ($CDCl_3$) δ (3^1R/3^1S=9/1)=11.09 (1H, s, CHO), 10.48/10.51, 9.58, 8.50 (each 1H, s, 5-, 10-, 20-H), 6.52 (1H, q, J=7 Hz, 3-CH), 5.24, 5.08 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.45–4.47 (1H, m, 18-H), 4.26–4.28 (1H, m, 17-H), 4.05 (2H, q, J=7 Hz, 12-CH_2), 3.94 (2H, t, J=7 Hz, 8-CH_2), 3.50, 3.49 (each 3H, s, 2-CH_3, $COOCH_3$), 2.20–2.70 (6H, m, 8^1-CH_2, 17-CH_2CH_2), 2.15 (3H, d, J=7 Hz, 3^1-CH_3), 1.94 (3H, t, J=7 Hz, 12^1-CH_3), 1.81 (3H, d, J=7 Hz, 18-CH_3), 1.21 (3H, t, J=8 Hz, 8^2-CH_3), −1.59 (1H, s, NH). MS (FAB) found: m/z 608. Calcd for $C_{36}H_{40}N_4O_5$: M^+, 608.

4.3.16. Methyl 8-isobutyl-12-ethyl-bacteriopheophorbide-*f* ([I,E]BPhe-f_M, 29). Oxidation of 7-(hydroxymethyl)chlorin **23** (1.5 mg, 2 μmol) with PDC gave **29** (0.7 mg, 46% yield, 3^1S only, rt=20 min); vis (CH_2Cl_2) λ_{max}=649 (rel. 21), 595 (6), 562 (6), 525 (7), 435 (100) nm; ^{1}H NMR ($CDCl_3$) δ=11.12 (1H, s, CHO), 10.57, 9.64, 8.50 (each 1H, s, 5-, 10-, 20-H), 6.55 (1H, q, J=7 Hz, 3-CH), 5.24, 5.09 (each 1H, d, J=20 Hz, 13^1-CH_2), 4.45–4.48 (1H, m, 18-H), 4.26–4.29 (1H, m, 17-H), 4.08 (2H, q, J=7 Hz, 12-CH_2), 3.90 (2H, d, J=8 Hz, 8-CH_2), 3.63, 3.44 (each 3H, s, 2-CH_3, $COOCH_3$), 2.26–2.77 (5H, m, 8^1-CH, 17-CH_2CH_2), 2.17 (3H, d, J=7 Hz, 3^1-CH_3), 1.95 (3H, t, J=7 Hz, 12^1-CH_3), 1.80 (3H, d, J=7 Hz, 18-CH_3), 1.24 (6H, t, J=8 Hz, 8^2-CH_3), −1.54 (1H, s, NH). MS (FAB) found: m/z 622. Calcd for $C_{37}H_{42}N_4O_5$: M^+, 622.

4.3.17. Methyl bacteriopheophorbides-*c*; [E,E]BPhe-c_M (30) and [P,E]BPhe-c_M (31). According to reported procedures,[19] *Chlorobium tepidum* gave two pure homologs [E,E]-**30** (3^1R only) and [P,E]-**31** (3^1R/3^1S=3/1) in the HPLC elution order.[22]

Compound **30**. Vis (CH_2Cl_2) λ_{max}=669 (rel. 45), 613 (6), 550 (13), 518 (8), 415 (100) nm; ^{1}H NMR ($CDCl_3$)[22] δ=9.94, 9.54 (each 1H, s, 5-, 10-H), 6.53–6.56 (1H, m, 3-CH), 5.26 (2H, s, 13^1-CH_2), 4.59 (1H, dq, J=2, 7 Hz, 18-H), 4.18–4.21 (1H, m, 17-H), 4.11 (2H, q, J=7 Hz, 12-CH_2), 3.89 (3H, s, 20-CH_3), 3.70 (2H, q, J=7 Hz, 8-CH_2), 3.58, 3.53, 3.30 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.58–2.62, 2.48–2.55 (each 2H, m, 17-CH_2CH_2), 2.16 (3H, d, J=7 Hz, 3^1-CH_3), 1.95 (3H, t, J=7 Hz, 12^1-CH_3), 1.70 (3H, t, J=8 Hz, 8^1-CH_3), 1.51 (3H, d, J=7 Hz, 18-CH_3), −1.79 (1H, s, NH). MS[25] (FAB) found: m/z 594. Calcd for $C_{36}H_{42}N_4O_4$: M^+, 594.

Compound **31**. Vis (CH_2Cl_2) λ_{max}=669 (rel. 45), 613 (6), 550 (13), 518 (8), 415 (100); ^{1}H NMR ($CDCl_3$)[22] δ (3^1R/3^1S=3/1)=9.95, 9.52 (each 1H, s, 5-, 10-H), 6.53–6.57 (1H, m, 3-CH), 5.26/5.25 (2H, s, 13^1-CH_2), 4.59 (1H, dq, J=2, 7 Hz, 18-H), 4.19–4.22 (1H, m, 17-H), 4.11 (2H, q, J=7 Hz, 12-CH_2), 3.89 (3H, s, 20-CH_3), 3.71 (2H, t, J=7 Hz, 8-CH_2), 3.58, 3.54, 3.30 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.60–2.65, 2.48–2.55 (each 2H, m, 17-CH_2CH_2), 2.16 (3H, d, J=7 Hz, 3^1-CH_3), 1.96 (3H, t, J=7 Hz, 12^1-CH_3), 1.83–1.87 (2H, m, 8^1-CH_2), 1.50 (3H, d, J=7 Hz, 18-CH_3), 1.23 (3H, t, J=7 Hz, 8^2-CH_3), −1.78 (1H, s, NH). MS[25] (FAB) found: m/z 608. Calcd for $C_{37}H_{44}N_4O_4$: M^+, 608.

4.3.18. 7,8-*cis*-Dihydroxy adduct of [E,E]BPhe-c_M (32). Oxidation of [E,E]BPhe-c_M **30** (38.0 mg, 0.064 mmol) with pyridine (0.627 ml) and OsO_4 (80.0 mg, 0.25 mmol) in dry CH_2Cl_2 (8.4 ml) for 3.5 h gave 7,8-*cis*-diol **32** (20.2 mg, 52% yield, 3^1R only, a 7/6 mixture of 7,8-stereoisomers); vis (CH_2Cl_2) λ_{max}=718 (rel. 35), 652 (12), 535 (35), 502 (11), 370 (100) nm; ^{1}H NMR ($CDCl_3$) δ (7/6)=8.81/8.80, 8.45/8.49 (each 1H, s, 5-, 10-H), 6.30–6.47 (1H, m, 3-CH), 4.92/4.76 (2H, s, 13^1-CH_2), 4.25–4.30 (1H, m, 18-H), 3.83–3.88 (1H, m, 17-H), 3.75–3.81 (2H, m, 12-CH_2), 3.64, 3.60/3.61, 3.37/3.39 (each 3H, s, 2-, 20-CH_3, $COOCH_3$), 2.29–2.54 (6H, m, 8-CH2, 17-CH_2CH_2), 2.05 (3H, d, J=7 Hz, 3^1-CH_3), 1.97/1.87 (3H, s, 7-CH_3), 1.79/1.76 (3H, t, J=7 Hz, 12^1-CH_3), 1.42/1.36 (3H, d, J=7 Hz, 18^1-CH_3), 1.20/1.10 (3H, t, J=8 Hz, 8^1-CH_3), −0.18/−0.07 (1H, s, NH). MS (FAB) found: m/z 628. Calcd for $C_{36}H_{44}N_4O_6$: M^+, 628.

4.3.19. 7,8-*cis*-Dihydroxy adduct of [P,E]BPhe-c_M (33). Oxidation of [P,E]BPhe-c_M **31** (64.8 mg, 0.11 mmol) with pyridine (0.98 ml) and OsO_4 (136 mg, 0.43 mmol) in dry CH_2Cl_2 (14.6 ml) for 4.5 h gave 7,8-*cis*-diol **33** (27.6 mg, 43% yield, 3^1R/3^1S=3/1, a 7/6 mixture of 7,8-stereoisomers); vis (CH_2Cl_2) λ_{max}=719 (rel. 35), 652 (12), 535 (35), 502 (12), 370 (100) nm; ^{1}H NMR ($CDCl_3$) δ=8.80/8.79/8.78, 8.51/8.50/8.46/8.44 (each 1H, s, 5-, 10-H), 6.30–6.37 (1H, m, 3-CH), 4.92/4.78 (2H, s, 13^1-CH_2), 4.25–4.30 (1H, m, 18-H), 3.83–3.90 (1H, m, 17-H), 3.78 (2H, q, J=7 Hz, 12-CH_2), 3.63, 3.61/3.60, 3.40/3.37 (each 3H, s, 2-, 20-CH_3, $COOCH_3$), 2.29–2.57 (6H, m, 8-CH_2, 17-CH_2CH_2), 2.05 (3H, d, J=7 Hz, 3^1-CH_3), 2.01/1.97 (3H, s, 7-CH_3), 1.82–1.89 (5H, m, 8^1-CH_2, 12^1-CH_3), 1.42/1.41 (3H, d, J=7 Hz, 18^1-CH_3), 1.06/1.10 (3H, t, J=8 Hz, 8^2-CH_3), −0.01/−0.19 (1H, s, NH). MS (FAB) found: m/z 642. Calcd for $C_{37}H_{46}N_4O_6$: M^+, 642.

4.3.20. 7^1-Hydroxy-[E,E]BPhe-c_M (34) and 8^1-hydroxy-[E,E]BPhe-c_M (36). Dehydration of 7,8-*cis*-diol **32** (16.3 mg, 26 μmol) with conc. HCl (320 μl) and H_2O (2.3 ml) in 1,4-dioxane (12 ml) for 1 min gave 7-(hydroxymethyl)chlorin **34** (2.0 mg, 12% yield, 3^1R only) and 8-(1-hydroxyethyl)chlorin **36** (13.2 mg, 81% yield, 3^1R only, 8^1R/8^1S=1/1).

Compound **34**. Vis (CH_2Cl_2) λ_{max}=656 (rel. 40), 610 (9), 555 (9), 520 (11), 418 (100) nm; ^{1}H NMR ($CDCl_3$) δ=10.12, 9.59 (each 1H, s, 5-, 10-H), 6.55 (1H, q, J=7 Hz, 3-CH), 5.80 (2H, s, 7-CH_2), 5.24 (2H, s, 13^1-CH_2), 4.56–4.59 (1H, m, 18-H), 4.17–4.20 (1H, m, 17-H), 4.10 (2H, q, J=7 Hz, 12-CH_2), 3.88 (3H, s, 20-CH_3), 3.71 (2H, q, J=8 Hz, 8-CH_2), 3.58, 3.52 (each 3H, s, 2-CH_3, $COOCH_3$), 2.46–2.50, 2.18–2.22 (each 2H, m, 17-CH_2CH_2), 2.16 (3H, d, J=7 Hz, 3^1-CH_3), 1.95 (2H, t, J=7 Hz, 12^1-CH_3), 1.76 (3H, t, J=8 Hz, 8^1-CH_3), 1.50 (3H, d, J=7 Hz, 18-CH_3), −1.80 (1H, s, NH). MS[26] (FAB) found: m/z 610. Calcd for $C_{36}H_{42}N_4O_5$: M^+, 610.

Compound **36**. Vis (CH_2Cl_2) λ_{max}=658 (rel. 42), 608 (11), 553 (9), 520 (9), 416 (100) nm; ^{1}H NMR ($CDCl_3$) δ (1/1)=9.99, 9.97/9.96 (each 1H, s, 5-, 10-H), 6.50–6.54 (1H, m, 3-CH), 6.25 (1H, q, J=7 Hz, 8-CH), 5.20/5.18 (2H, s, 13^1-CH_2), 4.56–4.58 (1H, m, 18-H), 4.13–4.15 (1H, m, 17-H), 4.10 (2H, q, J=7 Hz, 12-CH_2), 3.88 (3H, s, 20-CH_3), 3.59/3.58, 3.52/3.51, 3.37/3.36 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.54–2.61, 2.45–2.53 (each 2H, m, 17-CH_2CH_2), 2.13–2.16 (6H, m, 3^1-, 8^1-CH_3), 1.95 (2H, t, J=7 Hz, 12^1-CH_3), 1.48/1.46 (3H, d, J=7 Hz, 18-CH_3), −1.89/−1.93 (1H, s, NH). MS (FAB) found: m/z 610. Calcd for $C_{36}H_{42}N_4O_5$: M^+, 610.

4.3.21. 7^1-Hydroxy-[P,E]BPhe-c_M (35) and 8^1-hydroxy-[P,E]BPhe-c_M (37). Dehydration of 7,8-*cis*-diol **33** (11.8 mg, 18 μmol) with conc. HCl (309 μl) and H_2O (2.0 ml) in 1,4-dioxane (9 ml) for 2 min gave 7-(hydroxymethyl)chlorin **35** (1.2 mg, 10% yield, 3^1R/3^1S=3/1) and 8-(1-hydroxyethyl)chlorin **37** (7.3 mg, 61% yield, 3^1R/3^1S=3/1, 8^1R/8^1S=1/1).

Compound **35**. Vis (CH_2Cl_2) λ_{max}=656 (rel. 42), 610 (10), 555 (9), 520 (11), 418 (100) nm; ^{1}H NMR ($CDCl_3$) δ=10.15, 9.59 (each 1H, s, 5-, 10-H), 6.56–6.59 (1H, m, 3-CH), 5.80 (2H, s, 7-CH_2), 5.25 (2H, s, 13^1-CH_2), 4.59 (1H, dq, J=2, 7 Hz, 18-H), 4.19–4.21 (1H, m, 17-H), 4.11 (2H, q, J=7 Hz, 12-CH_2), 3.90 (3H, s, 20-CH_3), 3.78 (2H, q, J=8 Hz, 8-CH_2), 3.65, 3.59 (each 3H, s, 2-CH_3, $COOCH_3$), 2.72–2.88, 2.42–2.55 (each 2H, m, 17-CH_2CH_2), 2.17 (3H, d, J=7 Hz, 3^1-CH_3), 1.96 (2H, t, J=7 Hz, 12^1-CH_3), 1.70–1.76 (2H, m, 8^1-CH_2), 1.50 (3H, d, J=7 Hz, 18-CH_3), 1.33 (3H, t, J=7 Hz, 8^2-CH_3), −1.78 (1H, s, NH). MS[26] (FAB) found: m/z 624. Calcd for $C_{37}H_{44}N_4O_5$: M^+, 624.

Compound **37**. Vis (CH_2Cl_2) λ_{max}=658 (rel. 42), 608 (11), 553 (9), 520 (9), 417 (100) nm; ^{1}H NMR ($CDCl_3$) δ=10.0, 9.96/9.95 (each 1H, s, 5-, 10-H), 6.52 (1H, q, J=7 Hz, 3-CH), 6.25 (1H, t, J=7 Hz, 8-CH), 5.18 (2H, s, 13^1-CH_2), 4.55–4.57 (1H, m, 18-H), 4.13–4.15 (1H, m, 17-H), 4.07 (2H, q, J=7 Hz, 12-CH_2), 3.88 (3H, s, 20-CH_3) 3.61, 3.52/3.50, 3.36/3.35 (each 3H, s, 2-, 7-CH_3, $COOCH_3$), 2.63–2.72, 2.45–2.53 (each 2H, m, 17-CH_2CH_2), 2.14–2.19 (2H,

m, 8^1-CH_2), 2.04 (3H, d, J=7 Hz, 3^1-CH_3), 1.93 (2H, t, J=7 Hz, 12^1-CH_3), 1.80 (3H, t, J=7 Hz, 8^2-CH_3) 1.48/1.46 (3H, d, J=7 Hz, 18-CH_3), −1.85/−1.88 (1H, s, NH). MS (FAB) found: m/z 624. Calcd for $C_{37}H_{44}N_4O_5$: M^+, 624.

4.3.22. Methyl 8,12-diethyl-bacteriopheophorbide-*e* ([E,E]BPhe-e_M, 38). Oxidation of 7-(hydroxymethyl)-chlorin **34** (2.0 mg, 3 μmol) with PDC gave **38**[27] (1.2 mg, 56% yield, 3^1R only, rt=20 min); vis (CH_2Cl_2) λ_{max}=660 (rel. 18), 605 (6), 575 (6), 537 (8), 445 (100) nm; ^{1}H NMR ($CDCl_3$) δ=11.25 (1H, s, CHO), 10.76, 9.71 (each 1H, s, 5-, 10-H), 6.68 (1H, q, J=7 Hz, 3-CH), 5.23 (2H, s, 13^1-CH_2), 4.60 (1H, dq, J=2, 7 Hz, 18-H), 4.19 (1H, dt, J=7, 2 Hz, 17-H), 4.07–4.11 (4H, m, 8-, 12-CH_2), 3.89 (3H, s, 20-CH_3), 3.59, 3.58 (each 3H, s, 2-CH_3, $COOCH_3$), 2.49–2.58, 2.27–2.31 (each 2H, m, 17-CH_2CH_2), 2.20 (3H, d, J=7 Hz, 3^1-CH_3), 1.97 (3H, t, J=7 Hz, 12^1-CH_3), 1.85 (3H, t, J=8 Hz, 8^1-CH_3), 1.53 (3H, d, J=7 Hz, 18-CH_3), −1.54 (1H, s, NH). MS (FAB) found: m/z 608. Calcd for $C_{36}H_{40}N_4O_5$: M^+, 608.

4.3.23. Methyl 8-propyl-12-ethyl-bacteriopheophorbide-*e* ([P,E]BPhe-e_M, 39). Oxidation of 7-(hydroxymethyl)chlorin **35** (1.5 mg, 3 μmol) with PDC gave **39**[27] (0.8 mg, 52% yield, $3^1R/3^1S$=3/1, rt=21 min); vis (CH_2Cl_2) λ_{max}=660 (rel. 18), 605 (6), 575 (6), 537 (8), 445 (100) nm; ^{1}H NMR ($CDCl_3$) δ=11.17 (1H, s, CHO), 10.75, 9.63 (each 1H, s, 5-, 10-H), 6.65 (1H, q, J=7 Hz, 3-CH), 5.27 (2H, s, 13^1-CH_2), 4.60 (1H, dq, J=2, 7 Hz, 18-H), 4.19 (1H, dt, J=7, 2 Hz, 17-H), 4.09 (2H, q, J=8 Hz, 12-CH_2), 4.01 (2H, t, J=8 Hz, 8-CH_2), 3.87 (3H, s, 20-CH_3), 3.59, 3.56 (each 3H, s, 2-CH_3, $COOCH_3$), 2.45–2.65, 2.25–2.35 (each 2H, m, 17-CH_2CH_2), 2.25 (2H, m, 8^1-CH_2), 2.20 (3H, d, J=7 Hz, 3^1-CH_3), 1.95 (3H, t, J=8 Hz, 12^1-CH_3), 1.53 (3H, d, J=7 Hz, 18-CH_3), 1.33 (3H, t, J=8 Hz, 8^2-CH_3), −1.57 (1H, s, NH). MS (FAB) found: m/z 622. Calcd for $C_{37}H_{42}N_4O_5$: M^+, 622.

4.4. Data of zinc methyl bacteriopheophorbides (Zn-BPhes)

4.4.1. Zinc methyl 8-ethyl-12-methyl-bacteriopheophorbide-*d* (Zn-[E,M]BPhe-d_M, Zn-1).[10] $3^1R/3^1S$=1/1, rt=7.5 min; rt(R)=57 and rt(S)=62 min, separation ratio (R_S)=2.2 (MeOH/H_2O=3/1); vis (CH_2Cl_2) λ_{max}=647 (rel. 76), 601 (11), 554 (5), 513 (3), 422 (100) nm. MS (FAB) found: m/z 628. Calcd for $C_{34}H_{36}N_4O_4^{64}Zn$: M^+, 628.

4.4.2. Zinc methyl 8-ethyl-12-methyl-bacteriopheophorbide-*f* (Zn-[E,M]BPhe-f_M, Zn-2).[14] $3^1R/3^1S$=1/1, rt=10 min; rt(R)=117 and rt(S)=124 min, R_S=1.0 (MeOH/H_2O=2/1); vis (CH_2Cl_2) λ_{max}=632 (rel. 35), 585 (9), 450 (100) nm. MS (FAB) found: m/z 642. Calcd for $C_{34}H_{34}N_4O_5^{64}Zn$: M^+, 642.

4.4.3. Zinc methyl 8-ethyl-12-methyl-bacteriopheophorbide-*c* (Zn-[E,M]BPhe-c_M, Zn-3).[10] $3^1R/3^1S$=2/1, rt=8 min; rt(R)=65 and rt(S)=69 min, R_S=1.7 (MeOH/H_2O=3/1); vis (CH_2Cl_2) λ_{max}=658 (rel. 63), 612 (11), 575 (7), 527 (4), 427 (100) nm. MS (FAB) found: m/z 642. Calcd for $C_{35}H_{38}N_4O_4^{64}Zn$: M^+, 642.

4.4.4. Zinc methyl 8-ethyl-12-methyl-bacteriopheophorbide-*e* (Zn-[E,M]BPhe-e_M, Zn-4). $3^1R/3^1S$=2/1, rt=9 min; rt(R)=120 and rt(S)=142 min, R_S=3.0 (MeOH/H_2O=2/1); vis (CH_2Cl_2) λ_{max}=643 (rel. 30), 593 (10), 457 (100) nm. MS (FAB) found: m/z 656. Calcd for $C_{35}H_{36}N_4O_5^{64}Zn$: M^+, 656.

4.4.5. Zinc methyl 8,12-diethyl-bacteriopheophorbide-*f* (Zn-[E,E]BPhe-f_M, Zn-27). 3^1R only, rt=10 min; vis (CH_2Cl_2) λ_{max}=632 (rel. 35), 585 (9), 450 (100) nm. MS (FAB) found: m/z 656. Calcd for $C_{35}H_{36}N_4O_5^{64}Zn$: M^+, 656.

4.4.6. Zinc methyl 8-propyl-12-ethyl-bacteriopheophorbide-*f* (Zn-[P,E]BPhe-f_M, Zn-28). $3^1R/3^1S$=9/1, rt=10 min; vis (CH_2Cl_2) λ_{max}=632 (rel. 35), 585 (9), 450 (100) nm. MS (FAB) found: m/z 670. Calcd for $C_{36}H_{38}N_4O_5^{64}Zn$: M^+, 670.

4.4.7. Zinc methyl 8-isobutyl-12-ethyl-bacteriopheophorbide-*f* (Zn-[I,E]BPhe-f_M, Zn-29). 3^1S only, rt=10 min; vis (CH_2Cl_2) λ_{max}=632 (rel. 35), 585 (9), 450 (100) nm. MS (FAB) found: m/z 684. Calcd for $C_{37}H_{40}N_4O_5^{64}Zn$: M^+, 684.

Acknowledgements

We thank Mr Masanobu Kubo of Ritsumeikan University for his experimental assistance. This work was partially supported by Grants-in-Aid for Scientific Research (No. 14050096 and 15033271) on Priority Areas (417) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government.

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