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Syntheses and optical properties of stable 8-alkylidene-bacteriochlorins mimicking the molecular structures of natural bacteriochlorophylls-*b* and *g*

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Abstract—We prepared bacteriochlorophyll(BChl)-*b* and *g* models by Diels–Alder reactions of 8-vinyl-chlorophylls with tetracyanoethylene. The resulting 8-alkylidene-bacteriochlorins with various substituent groups at the 3-position had the same π-conjugate as BChls-*b*/*g*. While the natural pigments isomerized by addition of an acid to afford the corresponding chlorins, the synthetic models were stable under the acidic conditions due to dialkylation at the 7-position. These BChl-*b*/*g* models are useful for investigating the optical properties of relatively unstable BChls-*b*/*g*.

1. Introduction

Bacteriochlorophyll(BChl)s-*b* and *g* are found in some purple bacteria[1] and heliobacteria,[2] respectively, and their molecular structures including the absolute configurations have already been determined (left drawing of Fig. 1).[3,4] They function as photosynthetic pigments in natural light-harvesting, energy transfer and charge separation systems as do chlorophyll(Chl)s-*a*/*d* in higher plants or cyanobacteria and (Zn-)BChl-*a* in photosynthetic bacteria.[5] As compared with any other photosynthetic (B)Chls (Chls-*a*/*b*/*c*/*d*, BChls-*a*/*c*/*d*/*e*), BChls-*b* and *g* are quite unique in their molecular structures possessing an ethylidene group at the 8-position, and have the same bacteriochlorin π-conjugate (doubly saturated porphyrin moiety at 7–8 and 17–18 bonds). BChl-*b* possesses an acetyl group (R^3) at the 3-position, which is

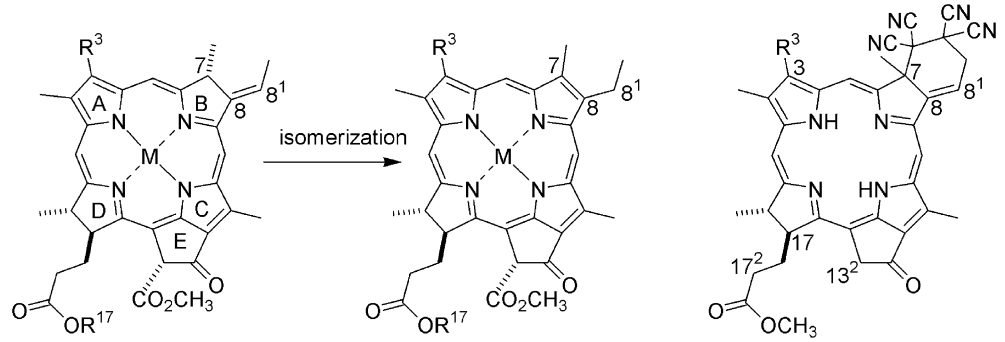

Figure 1. Molecular structures of natural BChls- and BPhes-*b*/*g* (left), their isomerized products (center) and synthetic stable model compounds **1**–**5** (right).

Keywords: Bacteriochlorin; Diels–Alder reaction; Isomerization; Tetracyanoethylene.
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the same function group as in BChl-*a*. BChl-*g* possesses a vinyl group (R^3) at the 3-position, which is the same substituent as in Chl-*a*. Only a few reports are available on in vitro investigation of BChls-*b*/*g*[6,7] because of their instabilities, i.e. the easy isomerization of the bacteriochlorin moiety (two reduced pyrroles at rings B and D) to the corresponding chlorin (one reduced pyrrole at ring D) even under ambient conditions as shown in Figure 1.[8,9] Stable bacteriochlorin models possessing a C8=C8^1 double bond are thus necessary to elucidate the optical properties of naturally occurring BChls-*b*/*g*. In this paper, we intended to synthesize chemically stable 8-alkylidene-bacteriochlorins **1**–**5** (right drawing of Fig. 1) as the BChl-*b*/*g* models.

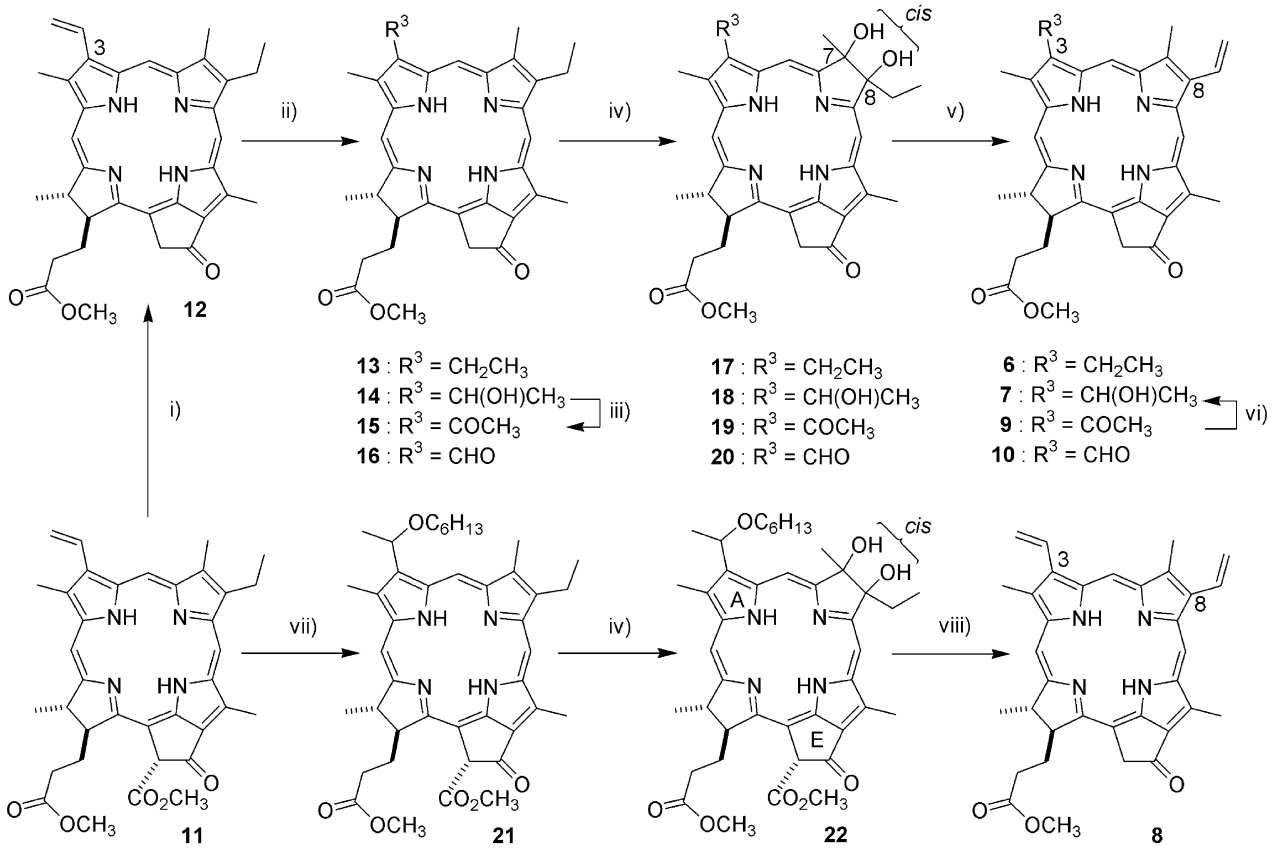
To form the C8=C8^1 double bond, we employed Diels–Alder reaction of 8-vinyl-chlorin with tetaracyanoethylene (TCNE). Various Diels–Alder reactions of cyclic tetrapyrroles have been studied.[10–15] In most cases, β-vinylporphyrins or chlorins, C(β′)=C(β)–CH=CH_2, reacted with a dienophile to give the corresponding [4+2] cycloadduct with one more reduced π-conjugate, –C(β′)–C(β)=CH–CH_2–. The resulting Diels–Alder products had a six-membered ring attached to the β,β′-positions and also an *exo*-double bond on the β-position similar to the 8-ethylidene group of BChls-*b*/*g*. In the chlorin π-conjugate system, the C=C double bond on the pyrrole ring opposite the reduced pyrolidine is more reactive than any other double bonds in the skeletal π-conjugate.[16] The Diels–Alder reaction of 8-vinyl-chlorophyll derivatives with a dienophile thus tended to give bacteriochlorin molecules bearing a C8=C8^1 double bond as models of BChls-*b*/*g* as in the right drawing of Figure 1. Additionally, the stereochemistry of the double bond was fixed as an *E*-form in the same stereoisomer with the natural BChls-*b*/*g*,[3,4] due to the C7–C8 fused six-membered ring. BChls-*b* and *g* have a phytyl, farnesyl or geranylgeranyl group as the 17-propionate ester and also have a methoxycarbonyl group at the 13^2-position. These moieties do not play any significant roles in their monomeric absorption spectra,[17–19] and are here changed to 17^2-$COOCH_3$ and 13^2-H_2 for preparing simple and stable model compounds.

Such Diels–Alder reactions would allow us to prepare a series of 8-alkylidene-BChl analogues possessing various substituents at the 3-position. Since the synthetic bacteriochlorins have two alkyl groups as peripheral substituents at the 7-position, undesired rearrangement of the bacteriochlorin moiety to the chlorin can no longer take place. Here, we report syntheses of stable bacteriochlorins possessing a similar π-conjugate system with natural BChls-*b*/*g* as well as their chemical stabilities and optical properties.

2. Results and discussion

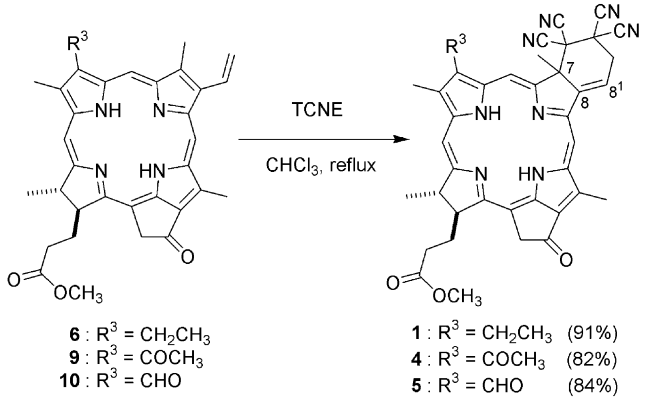
2.1. Synthesis of 8-vinyl-chlorophyll derivatives

As diene molecules, we prepared 8-vinyl-chlorins **6**–**10**



Scheme 1. Synthesis of 3-substituted 8-vinyl-chlorins: (i) collidine, reflux; (ii) H_2–Pd/C for **13**, 30% HBr–AcOH, H_2O, CH_2N_2 for **14**, cat. OsO_4–$NaIO_4$ in AcOH–THF for **16**; (iii) *N*-methyl-morphorine-*N*-oxide–Pr_4RuO_4; (iv) OsO_4-pyridine, H_2S; (v) TsOH, CH_2Cl_2–benzene (rt to reflux), CH_2N_2; (vi) $NaBH_4$–MeOH; (vii) 30% HBr–AcOH, 1-hexanol, CH_2N_2; (viii) 1,2-dichlorobenzene, 170 °C.

possessing various substituent groups at the 3-position as follows (Scheme 1). The 3-vinyl group of methyl pyropheophorbide-*a* (**12**)[20] was converted to ethyl, 1-hydroxyethyl and formyl groups (R^3) as in chlorins **13**,[20] **14**[21] and **16**,[20] respectively, according to reported procedures. Oxidation of 1-hydroxyethyl group in **14** produced 3-acetyl-chlorin **15**.[22] Reaction of chlorins **13–16** with OsO_4 in the presence of pyridine followed by treatment of H_2S gas[23–25] afforded the corresponding 7,8-*cis*-diols **17–20** in moderate yields. The resulting diols **17**, **19** and **20** were double-dehydrated by treatment of *p*-toluenesulfonic acid at room temperature followed by reflux[23,26,27] to give 3-ethyl, 3-acetyl and 3-formyl-8-vinyl-chlorins **6**, **9** and **10** as a major product. After the similar acidic treatment of **18**, 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** and 3,8-divinyl-chlorin **8** were produced in ~1 and 3%, respectively, with some degradation products. Desired **7** was successively prepared by reduction of the 3-acetyl group in **9**. To synthesize 3,8-divinyl-chlorin **8**, we utilized reported thermal degradation of **21**[28] derivatized from methyl pheophorbide-*a* **11**, because the β-keto ester moiety on the E-ring promoted an elimination reaction at the 3-position of the A-ring.[29] As described above, a series of 3-substituted 8-vinyl-chlorins **6–10** were prepared.

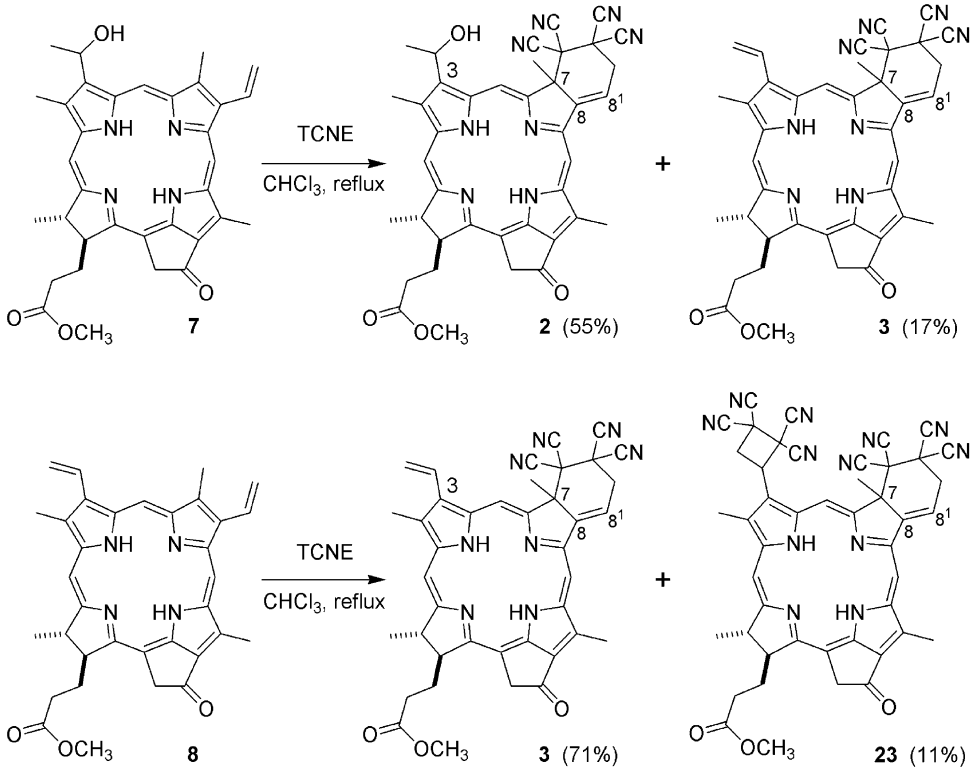


Scheme 2. Diels–Alder reactions of 3-ethyl-, 3-acetyl- and 3-formyl-8-vinyl-chlorins **6**, **9** and **10** with TCNE.

2.2. Diels–Alder reaction for preparing 8-alkylidene-BChl derivatives

Diels–Alder reactions of 8-vinyl-chlorins **6–10** as dienes with TCNE were achieved by the following procedures. A slight excess of TCNE (ca. 1.2 equiv) was added to **6–10** in dry chloroform and refluxed for 30 min under nitrogen. The Diels–Alder reaction was monitored by ultraviolet–visible–near infrared (UV–VIS–NIR) spectral analysis; a specific red-shift of the Qy absorption maximum based on a change of π-conjugate moieties from chlorin to bacteriochlorin. All the reactions afforded the corresponding 8-alkylidene-bacteriochlorins **1–5** as a 1:1 7-epimeric mixture. Molecular structures of all the Diels–Alder products **1–5** were determined by their 1D ^{1}H NMR, 2D ^{1}H–^{1}H correlation and rotating-frame Overhauser effect spectroscopies (COSY/ROESY) and high resolution mass (HR-MS, ionized by fast atomic bombardment (FAB)) spectral analyses: the specific proton signals of the 8-alkylidene group were situated around 7 and 4 ppm for 8-CH and 8^1-CH_2, respectively, and the main mass ion peak was observed at the position of one-to-one adduct.

In the reactions of 3-ethyl, 3-acetyl and 3-formyl-8-vinyl-chlorins **6**, **9** and **10** with TCNE, no significant side-reaction occurred and desired [4+2] cycloadducts **1**, **4** and **5** were



Scheme 3. Reactions of 3-(1-hydroxyethyl)-8-vinyl- and 3,8-divinyl-chlorins **7** and **8** with TCNE.

isolated in good yields after purification of flash column chromatography (FCC) and recrystallization (Scheme 2).

Diels–Alder reactions of **7** and **8** afforded the corresponding [4+2] cycloadducts **2** and **3**, respectively, and additional by-products (Scheme 3). Complete consumption of 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** led to the formation of desired **2** (55%) and its dehydrated product **3** (17%). In refluxing chloroform, neither 3-(1-hydroxyethyl)-8-ethyl-chlorin **14** in the presence of TCNE nor 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** in the absence of TCNE afforded their dehydrated chlorins **12** and **8**, but **2** gave the corresponding dehydrated **3**, indicating that thermal dehydration of 3-(1-hydroxyethyl) group occurred more easily in **2** than in **7** and **14**. Under the above reaction conditions, such a bacteriochlorin π-conjugate promoted elimination of a water molecule on the 3-position.

In the case of 3,8-divinyl-chlorin **8**, desired Diels–Alder adduct **3** was prepared predominantly after 30-min reflux. When the reaction mixture was worked up by a standard procedure, an undesired product was isolated besides **3** (71%). The over-reaction of **3** with TCNE occurred during evaporation of the reaction mixture in the dark to produce [2+2] cycloadduct **23** (11%).[11] Addition of excess TCNE (5 equiv) to the chloroform solution of **3** resulted in exclusive formation of **23**, but no [4+2] cycloadducts at around the 3-position could be isolated. In the chlorin chromophore (17,18-dihydroporphyrin), the 3-vinyl group essentially acted as an ene and the 8-vinyl group could function as a diene accompanying the C8═C7.

2.3. Chemical stabilities of synthetic models 3 and 4 in comparison with BPhes-*b*/*g*

Molecular structures and skeletal π-conjugates of synthetic **1–5** were quite similar to those of natural BChls-*b*/*g*, all of which had the C8═C8^1 double bond, so bacteriochlorins **1–5** might be model compounds for BChls-*b*/*g*. In this section, we will compare the chemical stabilities between the synthetic **3/4** and natural metal-free bacteriopheophytin(BPhe)s-*b*/*g* (see left drawing of Fig. 1).

Figure 2 shows UV–VIS–NIR spectral change of BPhes-*b*/*g* (upper) and the corresponding models **3/4** (lower) in acidic acetone in the dark. After acidic treatment, absorption peaks characteristic of BPhe-*b* at 777, 527 and 367 nm (red line of Fig. 2A) decreased with a concomitant increase in new peaks (blacks in Fig. 2A), and finally changed to peaks typical of chlorin chromophore at 674 and 421 nm (blue in Fig. 2A), which were similar to peaks of 3-acetyl-chlorin **15**. Normal-phase HPLC analysis showed that the products were a simple isomerized chlorin, 3-Ac-pheophytin(Phe)-*a* as a major product and its 8^1-oxidized chlorins (8^1-OH or 8^1-OOH, not determined)[6] as a minor product. These results indicated that the acidic treatment of metal-free BPhe-*b* promoted isomerization of C7H–C8═C8^1 to C7═C8–C8^{1}H moiety to give 3-Ac-Phe-*a* (Fig. 1) as in the

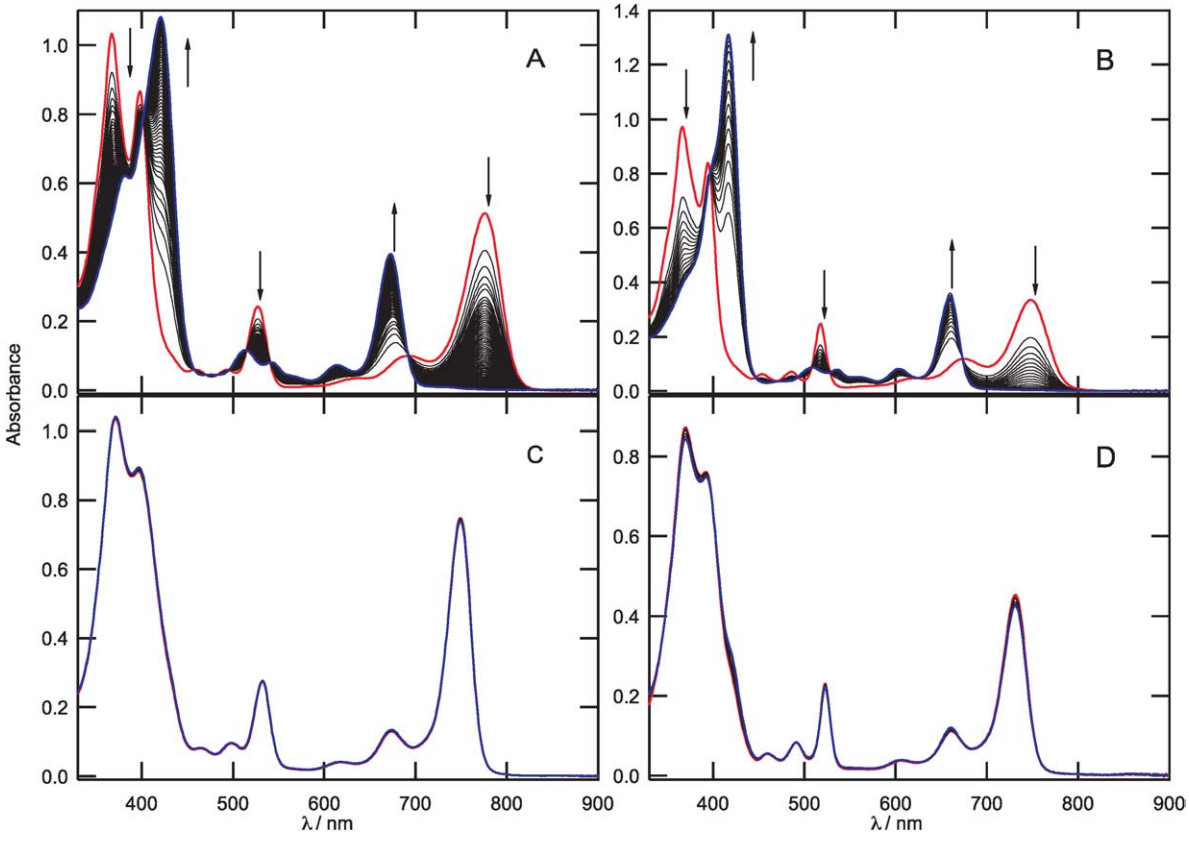


Figure 2. UV–VIS–NIR spectral changes of BPhe-*b* (A) and *g* (B), and the corresponding models **4** (C) and **3** (D) in acetone (3.0 ml) by acidic treatment (aqueous 3.5% HCl, 10 μl) at room temperature. Spectra were recorded at 3/2/30/30-min intervals for A/B/C/D, respectively. The red and blue lines are initial (before acid treatment) and final spectra (after 213/38/300/300-min treatment), respectively.

magnesium complex BChl-*b* (to 3-Ac-Chl-*a*) previously reported.[6,8]

Acidic treatment also induced isomerization of BPhe-*g* (absorption peaks at 748, 515 and 366 nm, red line of Fig. 2B) to a chlorin chromophore (660 and 417 nm, blue in Fig. 2B), resembling 3-vinyl-chlorin **12**. HPLC analyses supported that the major product was a chlorin chromophore possessing 660 nm as the Qy maxima. Therefore, BPhe-*g* isomerized by an action of an acid to give Phe-*a* (Fig. 1) as in BChl-*g* to Chl-*a*.[6,9] The isomerization of bacteriochlorin to chlorin chromophores was completed in 213 and 38 min for BPhes-*b* and *g*, respectively, and their half lifetimes were estimated to be 25 and 6 min, respectively, showing that the isomerization of BPhe-*g* was catalyzed by an acid more efficiently than that of BPhe-*b*, similar to the reported isomerization of BChls-*b*/*g*.[8,9]

We examined chemical stabilities of synthetic models **3** and **4** under the same acidic conditions (Fig. 2C and D). Almost no spectral changes were observed for 5 h. HPLC analyses after acidic treatment did not show any other peaks than **3/4**, indicating that synthetic models **3/4** were quite inactive for the acidic treatment compared to natural BPhes-*b*/*g*. Dialkylation of the 7-position and/or the fused ring on the 7,8-positions led to **3/4** being highly chemically stable.

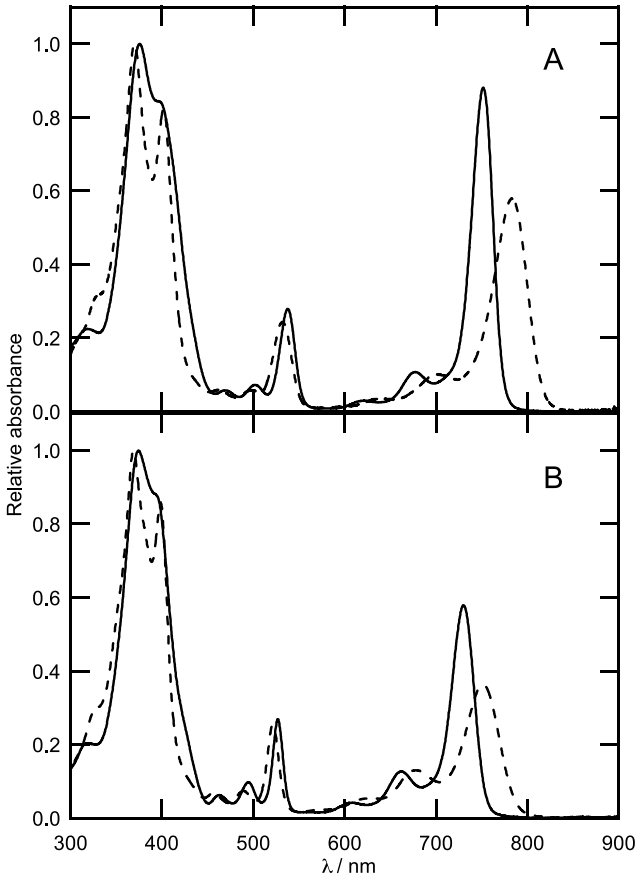


Figure 3. UV–VIS–NIR spectra of BPhe-*b* and **4** (A), and BPhe-*g* and **3** (B) in dichloromethane. Solid and broken lines were synthetic **4/3** and BPhes-*b*/*g*, respectively. All the spectra were normalized at the Soret peak.

2.4. Electronic absorption properties of BPhes-*b*/*g* and their synthetic stable models 1–5

Figure 3A shows UV–VIS–NIR spectra of 3-acetyl forms BPhe-*b* and **4** (broken and solid lines, respectively) in dichloromethane, and broken and solid lines in Figure 3B are spectra of 3-vinyl forms BPhe-*g* and **3**, respectively. Synthetic **4/3** had similar spectra to BPhes-*b*/*g* except for slight blue-shifted Qy maxima. In the Qy region (600–850 nm), all four bacteriochlorins had an intense Qy(0,0) band at a longer wavelength with association of a small Qy(1,0) band at a shorter wavelength. The Qy(0,0) maxima λ_{max}[Qy(0,0)]s of semi-natural BPhes-*b*/*g* were situated at 783/751 nm with full widths at half maximum (FWHMs) of 610/720 cm^{-1}, and those of synthetic **4/3** were situated at 752/730 nm with FWHMs of 430/500 cm^{-1}, respectively (see Table 1). BPhe-*b* and **4** possessing the 3-acetyl group had more red-shifted and sharper Qy(0,0) bands than BPhe-*g* and **3** which possessed the 3-vinyl group, respectively. The λ_{max}[Qy(1,0)]s of BPhes-*b*/*g* and **4/3** were located at 700/677 and 677/662 nm, respectively. Energetic difference Δ between Qy(0,0) and Qy(1,0) peaks of BPhes-*b*/*g* and **4/3** was 1490/1450 and 1460/1410 cm^{-1}, respectively, showing the Δs were slightly larger in BPhe-*b* and **4** than in BPhe-*g* and **3**. Relative intensities I_{rel}s (based on the Soret peak intensity) in Qy(0,0) peaks of BPhe-*b*/*g* and **4/3** were 0.58/0.36 and 0.88/0.58, and their Qy(1,0) peak intensities were 0.10/0.13 and 0.11/0.13, showing that BPhe-*b* and **4** had more intense Qy(0,0) and less intense Qy(1,0) bands than BPhe-*g* and **3**, respectively.

In the Qx absorption region (460–550 nm) of all the above four bacteriochlorins, large Qx(0,0) bands were associated with small Qx(1,0) bands at shorter wavelength. The λ_{max}[Qx(0,0)]s of BPhe-*b*/*g* and **4/3** situated at 532/521 and 538/527 nm with FWHMs of 850/570 and 620/460 cm^{-1}, respectively, while I_{rel}s in Qx(0,0) peaks of BPhes-*b*/*g* and **4/3** were 0.24/0.25 and 0.28/0.27. BPhe-*b* and **4** had more red-shifted and broadened Qx(0,0) bands than BPhe-*g* and **3**. The Δs between Qx(0,0) and Qx(1,0) of BPhe-*b* and **4** (1310 and 1330 cm^{-1}) were slightly larger than those of BPhe-*g* and **3** (1260 and 1250 cm^{-1}) as observed in Qy bands. The same substituent effect on 3-acetyl/vinyl groups was thus clearly observed in both Qy and Qx bands of semi-natural BPhes-*b*/*g* and synthetic **4/3**. As described above, BPhes-*b*/*g* were easily transferred to their chlorin chromophores, so their visible spectra were sensitive to the presence of a small amount of such chlorin impurities; typically, small Qy(1,0) peaks of BPhes-*b*/*g* are situated at around intense Qy(0,0) peaks of their altered chlorins. It is noteworthy that the present spectral analyses can be achieved in detail by using stable models containing no chlorin-type impurities.

Next, we measured UV–VIS–NIR spectra of other bacteriochlorins **1/2/5** possessing the same skeletal π-conjugate as BPhes-*b*/*g*. The λ_{max}[Qy(0,0)]s of **1–5** as well as their λ_{max}[Qx(0,0)]s were red-shifted in the order of **1**<**2**<**3**<**4**<**5** (see Table 1). These observed orders were reproduced by model calculation using ZINDO/S[30–32] (Table 2). All the synthetic bacteriochlorins **1–5** are applicable to the investigation of optical properties of a series of 3-substituted 8-alkylidene-bacteriochlorins as described above; they

Table 1. UV–VIS–NIR peak data of **1–5** and BPhes-*b*/*g* in CH_2Cl_2

Compound	λ_{max}/nm (I_{rel}[a]) [FWHM/cm^{-1}]		Δ[Qy(0,0)–(1,0)][b] /cm^{-1}	λ_{max}/nm (I_{rel}[a]) [FWHM/cm^{-1}]		Δ[Qx(0,0)–(1,0)][c] /cm^{-1}
	Qy(0,0)	Qy(1,0)		Qx(0,0)	Qx(1,0)	
1	715 (0.50) [490]	651 (0.13)	1370	522 (0.25) [450]	490 (0.10)	1250
2	722 (0.56) [480]	656 (0.13)	1410	524 (0.27) [460]	492 (0.10)	1260
3	730 (0.58) [500]	662 (0.13)	1410	527 (0.27) [460]	494 (0.10)	1250
4	752 (0.88) [430]	677 (0.11)	1460	538 (0.28) [620]	502 (0.07)	1330
5	760 (1.30) [340]	686 (0.09)	1430	543 (0.39) [520]	507 (0.08)	1290
BPhe-b	783 (0.58) [610]	701 (0.10)	1490	532 (0.24) [850]	497 (0.06)	1310
BPhe-g	751 (0.36) [720]	677 (0.13)	1450	521 (0.25) [570]	489 (0.07)	1260

[a] Relative peak intensity I_{rel} was based on the Soret peak intensity.
[b] $\Delta[\mathrm{Qy}(0,0)-(1,0)] = (1/\lambda_{max}[\mathrm{Qy}(1,0)] - 1/\lambda_{max}[\mathrm{Qy}(0,0)]) \times 10^7$.
[c] $\Delta[\mathrm{Qx}(0,0)-(1,0)] = (1/\lambda_{max}[\mathrm{Qx}(1,0)] - 1/\lambda_{max}[\mathrm{Qx}(0,0)]) \times 10^7$.

Table 2. Absorption maxima (nm) observed for **1–5** in CH_2Cl_2 (exp) and estimated by ZINDO/S calculation (calcd)

Compound	Qy(0,0)		Qx(0,0)	
	Exp	Calcd	Exp	Calcd
1	715	727	522	551
2	722	727	524	552
3	730	739	527	560
4	752	745	538	562
5	760	751	543	563

would also be useful to elucidate optical properties of semi-natural BPhes-*b*/*g*, as well as natural BChls-*b*/*g* in the distorted forms in the polypeptide environment where the 3-substituents were conformationally restricted for rotation around the 3–3[1] bond.[33,34]

3. Experimental

3.1. General

UV–VIS–NIR spectra were measured in air-saturated solvents at room temperature on a Hitachi U-3500 spectrophotometer. ^{1}H NMR spectra in chloroform-d were recorded at room temperature with a JEOL JNM-A400 Fourier transform NMR spectrometer; tetramethylsilane was used as an internal standard. ^{1}H–^{1}H COSY and ^{1}H–^{1}H ROESY (τ_m=400 ms) were recorded to determine the molecular structure of synthetic compounds. FAB-MS spectra were recorded on a JEOL GCmate II spectrometer; FAB-MS samples were dissolved in chloroform and *m*-nitrobenzyl alcohol and polyethylene glycol were used as a matrix and an internal standard, respectively. HPLC was carried out with a Shimadzu LC-10AD pump and an SPD-M10A photodiode array detector. A packed silica gel column (Cosmosil 5SL-II, Nacalai Tesque, 6.0ϕ 250 mm) was used for normal-phase HPLC. Dichloromethane and acetone for UV–VIS–NIR spectra were purchased from Nacalai Tesque (grade for spectroscopy). FCC was carried out on silica gel (Merck Kieselgel 60, 9358). All procedures including syntheses and spectral measurements were performed in the dark.

Methyl pheophorbide-*a* (**11**),[35] methyl pyropheophorbide-*a* (**12**),[20] methyl 3-devinyl-3-ethyl-pyropheophorbide-*a* (**13**),[20] methyl bacteriopheophorbide-*d* (**14**),[21] methyl 3-acetyl-3-devinyl-pyropheophorbide-*a* (**15**)[22] and methyl pyropheophorbide-*d* (**16**)[20] were prepared according to the reported procedures.

3.2. Estimation of electronic absorption peaks in 1–5 using ZINDO/S

Initial molecular structures of bacteriochlorins **1–5** were made using MM+ and PM3 in HYPERCHEM version 6.0 according to the literature.[30–32] In the calculation, configurations of the 7-methyl group in **1–5** were fixed in the same direction as natural BChls- and BPhes-*b*/*g*. Their energy minimized structures were made by repeating ZINDO/S and MM+ calculations until the MM+ calculation was finished with one cycle.[30] In the ZINDO/S calculation, were used HOMO and LUMO whose energy gaps were less than 7 eV. Their electronic absorption peaks were estimated from the ZINDO/S calculation based on their energy minimized structures.

3.3. Oxidation of C7–C8 double bond[23,25]

1.4 Equivalent of OsO_4 and a small amount of pyridine were added to a dichloromethane solution of chlorin (1 equiv). After stirring at room temperature for 16 h under nitrogen, the reaction was quenched by addition of methanol and 10-min bubbling of H_2S gas. The resulting OsS_4 was removed and the filtrate was evaporated in vacuo. The residue was purified by FCC and recrystallization from dichloromethane and hexane to give the corresponding 7,8-*cis*-dihydroxy-chlorin.

Oxidation of **13** gave **17** in 69% yield (lit.,[23] 52%). Oxidation of **14** gave **18** in 57% yield (lit.,[25] 56%). Oxidation of **15** gave **19** in 75% yield (lit.,[27] 57%). Oxidation of **16** gave **20** in 71% yield (lit.,[24] 74%). Oxidation of **21** gave **22**[28] and the crude product without FCC was used for the following pyrolysis due to instability

of **22** which possessed the 13^2-methoxycarbonyl group for FCC.

3.4. Preparation of 3-substituted 8-vinyl-chlorins

3.4.1. Double dehydration of 7,8-*cis*-diol[23,27]. To a dichloromethane and benzene solution (1:4) of 7,8-*cis*-diol, *p*-toluenesulfonic acid was added and the reaction mixture was stirred at room temperature for 2 h under nitrogen. After consumption of the diol was completed to give singly dehydrated 8-(1-hydroxyethyl)-chlorin by monitoring blue-shifted Qy maximum to 650–690 from 700–760 nm, the reaction mixture was refluxed for 30 min. The solution was poured into ice-cold water and extracted with dichloromethane. The organic phase was washed with 4% $KHSO_4$ and water, and dried over Na_2SO_4. After evaporation in vacuo, the residue was dissolved in a small amount of dichloromethane and subsequently an ethereal diazomethane solution was added and stirred for 30 min. After removal of the solvents in vacuo, the residue was purified with FCC and recrystallization from dichloromethane and hexane to give pure 8-vinyl-chlorin.

Dehydration of **17** gave **6** in 60% yield (lit.,[23] 40%). Dehydration of **19** gave **9** in 28% yield (lit.,[27] 44%). The other double dehydration of **22** including removal of hexanol and hydrolysis-de(carbon dioxide) of methoxycarbonyl group by heating in 1,2-dichlorobenzene at 160 °C was done according to the reported procedures[28] to give **8** in 40% total yield based on **21** (lit.,[28] 45%).

Compound **10**. Dehydration of **20** (52.0 mg) in dichloromethane and benzene (8/24 ml) afforded methyl 8-deethyl-8-vinyl-pyropheophorbide-*d* (**10**, 11.7 mg, 24%) as dark brown solid after FCC (3–4% Et_2O/CH_2Cl_2) and recrystallization (CH_2Cl_2/hexane); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=693$ (relative intensity, 0.60), 631 (0.07), 557 (0.09), 526 (0.14), 436 (1.00), 390 nm (0.60); ^{1}H NMR ($CDCl_3$) $\delta=11.57$ (1H, s, 3-CHO), 10.41 (1H, s, 5-H), 9.80 (1H, s, 10-H), 8.85 (1H, s, 20-H), 7.97 (1H, dd, $J=11$, 18 Hz, 8-CH), 6.65 (1H, d, $J=11$ Hz, 8^1-CH-*cis*-to 8-CH), 6.20 (1H, d, $J=18$ Hz, 8^1-CH_2-*trans*-to 8-CH), 5.35, 5.20 (each 1H, d, $J=19$ Hz, 13^1-CH_2), 4.59 (1H, dq, $J=2$, 8 Hz, 18-H), 4.49 (1H, dt, $J=8$, 2 Hz, 17-H), 3.79 (3H, s, 2-CH_3), 3.73 (3H, s, 12-CH_3), 3.62 (3H, s, $COOCH_3$), 3.46 (3H, s, 7-CH_3), 2.69–2.78, 2.56–2.65, 2.25–2.39 (1H+1H+2H, m, 17-CH_2CH_2), 1.85 (3H, d, $J=8$ Hz, 18-CH_3), −0.19 and −2.05 (each 1H, s, NH). MS (FAB) found: *m/z* 548.2417. Calcd for $C_{33}H_{32}N_4O_4$: M^+, 548.2424.

3.4.2. Methyl 8-deethyl-8-vinyl-bacteriopheophorbide-*d* (7).[27] To a dichloromethane solution (15 ml) of 3-acetyl-chlorin **8** (13.3 mg), a methanol solution (200 µl) saturated with $NaBH_4$ was added and the reaction mixture was stirred for 10 min under nitrogen. The reaction mixture was poured into ice-cold water and the separated organic phase was washed with 2% HCl and water, and dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified with FCC (8% Et_2O/CH_2Cl_2) and recrystallization from dichloromethane and hexane to give pure **7** (10.7 mg) in 80% yield (lit.,[27] 82%).

3.5. Diels–Alder reaction

TCNE (1.2 equiv) was added to a 8-vinyl-chlorin (ca. 50 µmol) in dry chloroform (25 ml) and refluxed for 30 min under nitrogen. The solution was poured into water, washed twice, extracted with dichloromethane and dried over Na_2SO_4. After evaporation, the residue was purified by FCC (Et_2O/CH_2Cl_2) and recrystallization from dichloromethane and hexane to give the corresponding bacteriochlorin as a 1:1 7-epimeric mixture.

3.5.1. TCNE adduct of 3-ethyl-8-vinyl-chlorin (1). Diels–Alder reaction of 3-ethyl-8-vinyl-chlorin **6** with TCNE afforded bacteriochlorin **1** as a dark green solid in 91% yield (3–4% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=715$ (rel. 0.50), 651 (0.13), 598 (0.04), 522 (0.25), 490 (0.10), 460 (0.06), 393 (0.85), 370 nm (1.0); ^{1}H NMR ($CDCl_3$) $\delta=8.76/75$ (1H, s, 10-H), 8.62/61 (1H, s, 5-H), 8.27/26 (1H, s, 20-H), 6.74–6.79 (1H, m, 8-CH), 5.05/4.89, 5.02/4.86 (each 1H, d, $J=20$ Hz, 13^1-CH_2), 4.26 (1H, m, 18-H), 4.08 (1H, m, 17-H), 3.82–3.99 (2H, m, 8^1-CH_2), 3.64–3.68 (2H, m, 3-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.43 (3H, s, 12-CH_3), 3.17 (3H, s, 2-CH_3), 2.48–2.58, 2.17–2.33 (each 2H, m, 17-CH_2CH_2), 2.24/23 (3H, s, 7-CH_3), 1.67–1.74 (3H+3H, m, 3^1-CH_3, 18-CH_3), 0.64/62 and −0.69/71 (each 1H, s, NH). MS (FAB) found: *m/z* 676.2920. Calcd for $C_{40}H_{36}N_8O_3$: M^+, 676.2910.

3.5.2. TCNE adduct of 3-(1-hydroxyethyl)-8-vinyl-chlorin (2). Diels–Alder reaction of 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** with TCNE afforded bacteriochlorin **2** as a 3^1/7-diastereomeric mixture as a green solid in 55% yield (5–6% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=722$ (rel. 0.56), 656 (0.13), 598 (0.04), 524 (0.27), 492 (0.10), 461 (0.06), 394 (0.87), 371 nm (1.0); ^{1}H NMR ($CDCl_3$) $\delta=9.33/32/19/15$ (1H, s, 5-H), 8.82/81 (1H, s, 10-H), 8.36/35/34/33 (1H, s, 20-H), 6.71–6.80 (1H, m, 8-CH), 6.23–32 (1H, m, 3-CH), 5.02–5.14, 4.87–4.93 (each 1H, m, 13^1-CH_2), 4.29 (1H, m, 18-H), 4.11 (1H, m, 17-H), 3.81–3.99 (2H, m, 8^1-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.49/48/45 (3H, s, 12-CH_3), 3.30/29/27/26 (1H, s, 2-CH_3), 2.49–2.63, 2.17–2.32 (each 2H, m, 17-CH_2CH_2), 2.25/24/23 (3H, s, 7-CH_3), 2.08–2.15 (3H, m, 3^1-CH_3), 1.67–1.75 (3H, m, 18-CH_3), 0.36/33 and −0.96/99 (each 1H, s, NH). MS (FAB) found: *m/z* 692.2888. Calcd for $C_{40}H_{36}N_8O_4$: M^+, 692.2860.

3.5.3. TCNE adduct of 3,8-divinyl-chlorin (3). Diels–Alder reaction of 3,8-divinyl-chlorin **8** with TCNE afforded bacteriochlorin **3** as a dark green solid in 71% yield (3–4% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=730$ (rel. 0.58), 662 (0.13), 608 (0.04), 527 (0.27), 494 (0.10), 463 (0.06), 394 (0.88), 374 nm (1.0); ^{1}H NMR ($CDCl_3$) $\delta=$ 8.83 (1H, s, 5-H), 8.82 (1H, s, 10-H), 8.40 (1H, s, 20-H), 7.75–7.80 (1H, m, 3-CH), 6.77 (1H, m, 8-CH), 6.19–6.27 (2H, m, 3^1-CH_2), 5.03–5.10, 4.88–4.94 (each 1H, m, 13^1-CH_2), 4.30 (1H, m, 18-H), 4.12 (1H, m, 17-H), 3.82–3.98 (2H, m, 8^1-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.46 (3H, s, 12-CH_3), 3.29 (3H, s, 2-CH_3), 2.50–2.66, 2.21–2.40 (each 2H, m, 17-CH_2CH_2), 2.24/2.23 (3H, s, 7-CH_3), 1.74/71 (3H, d, $J=8$ Hz, 18-CH_3), 0.34/32 and −0.91/93 (each 1H, s, NH). MS (FAB) found: *m/z* 674.2765. Calcd for $C_{40}H_{34}N_8O_3$: M^+, 674.2754.

3.5.4. TCNE adduct of 3-acetyl-8-vinyl-chlorin (4). Diels–Alder reaction of 3-acetyl-8-vinyl-chlorin **9** with TCNE afforded bacteriochlorin **4** as a dark brown solid in 82% yield (3–4% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) λ_{max}=751 (rel. 0.88), 677 (0.11), 620 (0.03), 538 (0.28), 502 (0.07), 469 (0.06), 376 nm (1.0); ^{1}H NMR ($CDCl_3$) δ=9.63 (1H, s, 5-H), 8.99/98 (1H, s, 10-H), 8.71/70 (1H, s, 20-H), 6.83–6.88 (1H, m, 8-CH), 5.13–5.21, 4.98–5.05 (each 1H, m, 13^1-CH_2), 4.41 (1H, m, 18-H), 4.22 (1H, m, 17-H), 3.83–4.02 (2H, m, 8^1-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.57 (3H, s, 2-CH_3), 3.53 (3H, s, 12-CH_3), 3.28 (3H, s, 3-$COCH_3$), 2.52–2.68, 2.18–2.34 (each 2H, m, 17-CH_2CH_2), 2.29 (3H, s, 7-CH_3), 1.74–1.79 (3H, d, J=8 Hz, 18-CH_3), −0.37/41 and −1.54/56 (each 1H, s, NH). MS (FAB) found: m/z 690.2696. Calcd for $C_{40}H_{34}N_8O_4$: M^+, 690.2703

3.5.5. TCNE adduct of 3-formyl-8-vinyl-chlorin (5). Diels–Alder reaction of 3-formyl-8-vinyl-chlorin **10** with TCNE afforded bacteriochlorin **5** as a dark brown solid in 84% yield (3–5% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) λ_{max}=760 (rel. 1.30), 686 (0.09), 627 (0.03), 543 (0.39), 507 (0.08), 474 (0.07), 381 nm (1.0); ^{1}H NMR ($CDCl_3$) δ=11.44 (1H, s, 3-CHO), 9.98/97 (1H, s, 5-H), 9.04/03 (1H, s, 10-H), 8.79/78 (1H, s, 20-H), 6.89–6.93 (1H, m, 8-CH), 5.17–5.25, 5.03–5.10 (each 1H, m, 13^1-CH_2), 4.44 (1H, m, 18-H), 4.26 (1H, m, 17-H), 3.87–4.04 (2H, m, 8^1-CH_2), 3.70 (3H, s, 2-CH_3), 3.65/63 (3H, s, $COOCH_3$) 3.56 (3H, s, 12-CH_3), 2.56–2.70, 2.18–2.30 (each 2H, m, 17-CH_2CH_2), 2.32 (3H, s, 7-CH_3), 1.77/80 (3H, d, J=8 Hz, 18-CH_3), −0.50/54 and −1.58/60 (each 1H, s, NH). MS (FAB) found: m/z 676.2564. Calcd for $C_{39}H_{32}N_8O_4$: M^+, 676.2547.

3.5.6. [2+2] TCNE adduct at the 3-vinyl group of 3 (23). The title compound **23** was isolated in 11% yield as a by-product in the Diels–Alder reaction of 3,8-divinyl-chlorin **8** with TCNE described above. Alternative preparation of **23** as a major product was achieved as follows. TCNE (3.0 equiv) was added to 3,8-divinyl-chlorin **8** (50 μmol) in dry chloroform (20 ml) and refluxed for 30 min under nitrogen. The solution was repeatedly evaporated in vacuo after addition of chloroform (each 15 ml). When the Qy maximum was completely red-shifted (730–740 nm), the reaction mixture was worked up in a similar manner with the above Diels–Alder reaction to give pure **23** as a 3^1/7-diastereomeric mixture as a dark brown solid in 78% yield (6% Et_2O/CH_2Cl_2 for FCC). The mixture was separated into two fractions on normal-phase silica gel HPLC (acetone/hexane=3:7) to afford each 3^1-epimerically pure sample as a 7-epimeric mixture, but the absolute configurations could not be determined. Fraction 1 (first elution): UV–VIS–NIR (CH_2Cl_2) λ_{max}=743 (rel. 1.23), 671 (0.14), 614 (0.06), 529 (0.36), 497 (0.12), 465 (0.09), 397 (1.0), 374 nm (0.91); ^{1}H NMR ($CDCl_3$) δ=9.10 (1H, s, 10-H), 8.96/92 (1H, s, 5-H), 8.77/76 (1H, s, 20-H), 6.92–6.99 (1H, m, 8-CH), 6.04–6.11 (1H, m, 3-CH), 5.17–5.29, 5.03–5.12 (each 1H, m, 13^1-CH_2), 4.94–5.04, 4.06–4.15 (each 1H, m, 3^1-CH_2), 4.47 (1H, m, 18-H), 4.27 (1H, m, 17-H), 3.88–4.07 (2H, m, 8^1-CH_2), 3.65/64 (3H, s, 2-CH_3), 3.62 (3H, s, $COOCH_3$) 3.57 (3H, s, 12-CH_3), 2.54–2.72, 2.16–2.38 (each 2H, m, 17-CH_2CH_2), 2.30 (3H, s, 7-CH_3), 1.81/77 (3H, d, J=7 Hz, 18-CH_3), −0.76/78 and −1.78/80 (each 1H, s, NH). Fraction 2 (second elution): UV–VIS–NIR (CH_2Cl_2) λ_{max}=743 (rel. 1.23), 671 (0.14), 614 (0.06), 529 (0.36), 497 (0.12), 465 (0.09), 397 (1.0), 374 nm (0.91); ^{1}H NMR ($CDCl_3$) δ=9.10 (1H, s, 10-H), 8.99/98 (1H, s, 5-H), 8.76 (1H, s, 20-H), 6.94–7.01 (1H, m, 8-CH), 6.28–6.38 (1H, m, 3-CH), 5.18–5.27, 5.04–5.13 (each 1H, m, 13^1-CH_2), 4.77–4.85, 4.02–4.07 (each 1H, m, 3^1-CH_2), 4.45 (1H, m, 18-H), 4.27 (1H, m, 17-H), 3.78–4.09 (2H, m, 8^1-CH_2), 3.67 (3H, s, 2-CH_3), 3.64/63 (3H, s, $COOCH_3$) 3.57 (3H, s, 12-CH_3), 2.53–2.71, 2.18–2.36 (each 2H, m, 17-CH_2CH_2), 2.37/36 (3H, s, 7-CH_3), 1.82/77 (3H, d, J=7 Hz, 18-CH_3), −0.58/61 and −1.62/64 (each 1H, s, NH). MS (FAB) found: m/z 802.2884 Calcd for $C_{46}H_{34}N_{12}O_3$: M^+, 802.2877.

3.6. Preparation of BPhes-*b*/*g*

An acetone solution (10 ml) of extracted pigments from cultured *Blastochloris viridis*[1] was demetallated by 5-min stirring with an aqueous diluted HCl solution (3 ml). The reaction mixture was poured into ice-cold water and extracted by dichloromethane. After evaporation in vacuo, the crude mixture including BPhe-*b* and some carotenoids was separated and purified by normal-phase HPLC (acetone/hexane=1/4) to give pure BPhe-*b*.

Pure BPhe-*g* was also prepared by the above procedures except that pigment extracts from *Heliobacterium modesticaldum* were used.[36]

3.7. Acidic treatment of natural BPhes-*b*/*g* and synthetic 4/3

To an acetone solution (3 ml) of sample (ca. 10 μmol), an aqueous 3.5% HCl solution (10 μl) was added allowed to stand in the dark. UV–VIS–NIR spectra were recorded at 2/3 min intervals for BPhes-*b*/*g* and every 30 min for **4** and **3**. When Qy maxima of BPhes-*b*/*g* had completely disappeared, the acidic acetone solution was diluted with water and extracted with dichloromethane. In **4/3**, the same work-up was done after 5-h standing. After evaporation in vacuo, the residue was analyzed by normal-phase HPLC (acetone/hexane=1/4 for BPhes-*b*/*g* and 3/7 for **4/3**). Chromatograms given from BPhe-*b* or *g* showed some chlorin chromophores, 3-Ac-Phe-*a* or Phe-*a* at 11 or 9 min, respectively, which was eluted faster than BPhe-*b* or *g* at 13 or 11 min, and a small amount of 8^1-oxidized chlorin at over 50 min. HPLC from **4** or **3** showed a single peak at 10 or 8 min, which was consistent with the elution time of **4** or **3**.

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