Tetrahedron 73 (2017) 313-321

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of monovinyl- and divinyl-chlorophyll analogs and their physical properties



Tetrahedro

Kifa Kim^a, Kazuki Tsuji^{a, b}, Yusuke Kinoshita^a, Tomohiro Miyatake^b, Hitoshi Tamiaki^{a, *}

^a Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

^b Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan

ARTICLE INFO

Article history: Received 16 November 2016 Received in revised form 2 December 2016 Accepted 5 December 2016 Available online 8 December 2016

Keywords: Fluorescence emission data Photosynthesis Pigment Pyropheophorbide Visible absorption spectra

ABSTRACT

Chlorophyll-*a* was chemically modified to methyl pyropheophorbides-*a* possessing 3,8-diethyl, 3-vinyl-8-ethyl, 3-ethyl-8-vinyl, and 3,8-divinyl groups. Analogous 3-ethyl-7-vinyl- and 3,7-divinyl-chlorins were prepared by derivatization of chlorophyll-*b*. The synthetic free bases as well as zinc 3-vinyl-chlorins were dissolved in THF and the monomeric diluted solutions were characterized by optical spectroscopy including visible absorption, circular dichroism, and fluorescence emission spectra. The optical data indicated that the 3-vinylation bathochromically shifted the visible (Soret/Qx/Qy) absorption and fluorescence emission maxima, the 7-vinylation moved the Soret/Qx and Qy/emission bands to longer and slightly shorter wavelengths, respectively, and the 8-vinylation induced red shifts of the Soret/Qx maxima and no (or faint red) shifts of the Qy/emission maxima. Zinc complexes of 3,7- and 3,8-divinylchlorins showed almost the same optical properties including fluorescence emission quantum yields and lifetimes as well as the same first oxidation potentials, thus, 3,7-divinyl-chlorophyll-*a* could be considered an alternative pigment to the naturally occurring 3,8-divinyl analog.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Phototrophs have several pigments including cyclic tetrapyrrole chlorophylls (Chls), linear tetrapyrrole bilins, and polyene carotenoids.^{1,2} In the initial stage of photosynthesis, Chls are important for light absorption, excitation energy migration, and charge separation processes. Most Chl molecules in oxygenic photosynthetic organisms have a vinyl group at the 3-vinyl group (left drawing of Fig. 1) except Chl-*d* bearing the 3-formyl group in the limited cyanobacterial species, such as *Acaryochloris marina*.^{3–5} One more vinyl group is found at the 8-position of the above 3-monovinylated Chl molecules in some natural cells to give 3,8-divinylated Chl molecules: Chl-*a* \rightarrow divinyl(DV)-Chl-*a* (*Synechococcus*/*Prochlorococcus*,^{6,7} Chl-*b* \rightarrow DV-Chl-*b* (*Prochlorococcus*,⁹ and

monovinyl(MV)-Chl- $c_3 \rightarrow$ Chl- c_3 (*Emiliania huxleyi*),¹⁰ while DV-Chl-*f* has not yet been found. Substitution of the 8-ethyl group with the 8-vinyl group shifts the intense absorption band on the blue side of the visible light region to a longer wavelength and induces no (or only slight) change of the redmost maximum.^{3,10,11} The former primarily controls the sunlight-harvesting ability and the latter regulates the site energy in photosynthetic apparatuses.

The 3-vinyl-8-ethyl-Chl molecules are biosynthesized through hydrogenation of the 8-vinyl group by specific enzymes, divinyl reductases.¹² Suppression of the ability of the reductase gives 3,8-divinylated analogs. As photosynthetically active monovinylated Chl pigments, regioisomeric 3-ethyl-8-vinyl-Chls are not detected in oxygenic phototrophs. No 3,8-diethyl-Chls are utilized in the initial events of photosynthesis. Furthermore, the 7-substituent of Chls- $a/c_1/c_2/d/f$ is a methyl group, while a formyl or methoxycarbonyl group is found in (DV-)Chl-b or (MV-)Chl-c₃, respectively.^{3,4,10} The functional groups at the 7-position are limited to the three substitutes and 7-vinylated Chls have not been observed. To address the reasons for the inaccessibility of such Chl molecules, we prepared diethylated, monovinylated, and divinylated Chl-*a*/*b* derivatives, **1a**, **1b**/**1c**/(Zn-)**2a**, and (Zn-)**2b**/ (Zn-)2c (right drawing of Fig. 1), and compared their optical and electrochemical properties in THF solution.



Abbreviations: APCI, atmospheric pressure chemical ionization; BChl, bacteriochlorophyll; Chl, chlorophyll; CD, circular dichroism; DV, divinyl; FCC, flash column chromatography; FWHM, full width at half maximum; HRMS, high resolution mass spectrum; LDI, laser desorption/ionization; MV, monovinyl; PDC, pyr-idinium dichromate; 7V, 7-vinyl.

Corresponding author.

E-mail address: tamiaki@fc.ritsumei.ac.jp (H. Tamiaki).



Fig. 1. Molecular structures of natural [divinyl(DV)-]chlorophylls(Chls)-a (left) and their synthetic analogs (right).

2. Results and discussion

2.1. Synthesis of (zinc) methyl pyropheophorbides

Chl-*a* from cyanobacterial cells (spirulina) was modified to methyl pyropheophorbide-*a* (**2a**, Scheme 1) according to reported procedures.^{5,13} The 3-vinyl group of **2a** was catalytically hydroge-nated to give methyl mesopyropheophorbide-*a* (**1a**), as shown in step (i) of Scheme 1.^{13,14} The C7=C8 double bond of **1a** was selectively oxidized to afford *cis*-diol **1d** [step (ii)], which was doubly dehydrated to yield 8-vinyl-chlorin **1c** [step (iii)].^{5,15}

The 3-vinyl substituent in **2a** was hydrated to a 3¹-epimeric mixture of the corresponding secondary alcohol, methyl 8-ethyl-12-methyl-bacteriopheophorbide-d ([E,M]BPhe-d_M) [step (iv) of Scheme 1]. The carbinol was also obtained from chemical alteration of extracted bacteriochlorophyll(BChl)-a or an isolated BChl-d homolog.¹³ Similarly to the synthesis of **1d** from **1a**, triol **2d** was obtained by the oxidation of $[E,M]BPhe-d_M^{15}$ [step (ii)] and was treated with an acid, *p*-toluenesulfonic acid [step (v)]. After reflux in THF for 1 h, bacteriochlorin 2d was transformed to chlorin compounds through monodehydration of the *cis*-7,8-diol.¹⁶ The crude alcoholic products were directly heated in 1,2dichlorobenzene under neutral conditions (reflux for 5 h) to induce further dehydration and afford 3,8-divinyl-chlorin (2c, methyl divinyl-pyropheophorbide-*a*) in a yield of 33% for the triple dehydration (Scheme S1). The molecular structure of 2c was characterized by its spectral data (section 4.2.3), which was also confirmed by the reported data.¹⁷ The one-pot simple conversion of 2d to 2c proceeded by refluxing the mixed solvents (1:9 $CH_2Cl_2-C_6H_6$) with the same acid¹⁸ but the isolated yield was lower (9%) than the above. Moreover, the Wittig reaction (vide infra)¹⁹⁻¹ of 3-formyl-8-vinyl-chlorin, methyl 8-vinyl-pyropheophorbide-d,¹⁵ with $CH_2 = PPh_3$ gave **2c** (46%). The transformation of **2a** to **2c** using the Wittig reaction took four steps including the oxidative cleavage of the 3-vinyl to formyl group and the overall yield was 6% (Scheme S2). The synthetic process through the triple dehydration [step (v)] is superior since it could be shortened to three steps and the total yield of 2a to 2c via 2d increased twofold (13%). Alternatively, 2c could be prepared by chemical modification of DV-Chl-a isolated from genetically mutated cyanobacterial cells^{9,24} based on the same procedures as in the aforementioned transformation of Chl-a to 2a.

Chl-*b* from spinach was altered to methyl 7¹-hydroxy-pyropheophorbide-*a* (2f)⁵ and oxidized by pyridinium dichromate (PDC) to give methyl pyropheophorbide-*b* (2e) in a yield of 55% [step (vi) of Scheme 1]. 7-Formyl-chlorin **2e** was treated with methylenetriphenylphosphorane prepared in situ from methyltriphenylphosphonium iodide and potassium tert-butoxide^{5,23} to afford 3,7-divinyl-chlorin **2b** [42%, step (vii)]. The new compound was identified by its ¹H NMR, mass, and visible spectra (section 4.2.2). According to previously reported procedures,⁵ the 3-vinyl group of 2f was selectively hydrogenated despite the presence of the reactive 7-hydroxymethyl group to 1f [step (viii)] and oxidized by PDC to 1e [step (vi)], then methylenated by the Wittig reaction to 1b [step (vii)]. Catalytic hydrogenation of 3,7divinyl-chlorin 2b gave a mixture of 3-vinyl-chlorin 2a and 7vinyl-chlorin 1b as the monohydrogenated products and 1a as the doubly hydrogenated product. No regioselective reduction occurred and the isolation of 1b from the reaction mixture was difficult. The non-selective hydrogenation was comparable to the less-selective hydrogenation (using rhodium on alumina as the catalyst) of DV-Chl-a reported previously.²⁵ It is noteworthy that the enzymatic reduction of divinyl-chlorophyllide-a (the dephytylated derivative of DV-Chl-a) proceeds regioselectively at the 8vinyl group to give solely the product monovinylated at the 3position, chlorophyllide-a.¹

Using the standard procedures shown in step (ix) of Scheme 2, free bases **2a**–**c** were metalated to zinc complexes Zn-**2a**–**c**. Synthetic Zn-**2a** and Zn-**2c** are models of natural Chl-*a* and DV-Chl-*a*, respectively, although the former two complexes lack the 13²-methoxycarbonyl group and are substituted at the central metal (Mg \rightarrow Zn) and esterifying group (phytyl \rightarrow methyl). Zn-**2b** is an analog of DV-Chl-*a*.

2.2. Optical properties of methyl mesopyropheophorbides **1a**-c

When **1a** was dissolved in THF, the diluted solution (ca. 10 μ M) gave apparently five absorption peaks in the visible region (Fig. 2A). The intense band in the purple light region is called a Soret band, the weak bands in the blue to green region are Qx bands, and the bands in the yellow to red region are Qy bands. All three visible bands are composed of the main band at a longer wavelength and the minor bands at shorter wavelengths. The Qx and Qy bands afford apparently two maxima, (0,0) and (0,1), as shown in Table 1, while the Soret band has two shoulders in the blue side of its main maximum at 407 nm.

Substitution of the 7-methyl group of **1a** with the vinyl group, as in **1b**, induced a slight blue shift of the Qy bands (1 nm) and redshift of the Soret band by 10 nm. The substitution largely affected the shape and positions of the Qx bands. The relative Qx(0,0) band intensity of **1b** halved in comparison with that of **1a**. The Qx(0,0)/



Scheme 1. Synthesis of methyl pyropheophorbides lacking a vinyl group 1a and possessing one vinyl group 1b/1c/2a and two vinyl groups 2b/2c: (i) H₂, Pd-C/THF, Me₂CO; (ii) OsO₄, C₅H₅N/CH₂Cl₂, then H₂S/MeOH; (iii) *p*-MeC₆H₄SO₃H·H₂O/C₆H₆ (rt \rightarrow reflux); (iv) HBr/AcOH, then H₂O, followed by CH₂N₂/Et₂O, CH₂Cl₂; (v) *p*-MeC₆H₄SO₃H·H₂O/THF (reflux), then 1,2-Cl₂C₆H₄ (reflux) (vi) (C₅H₅NH⁺)₂Cr₂O⁷/CH₂Cl₂; (vii) CH₃PPh₃⁺I⁻, tBuOK/CH₂Cl₂; (viii) H₂, Pd-Fibroin/THF, Me₂CO.

(0,1) maxima of **1b** moved to wavelengths of about 5 nm longer than those of **1a**. These spectral changes can be explained as follows. The Qx bands are largely regulated by the transition moment along the molecular x-axis, the B–D ring direction (Fig. 1), while the moment on the y-axis (A–C ring direction) perpendicular to the x-axis primarily controls the Qy bands. Therefore, the π -conjugatable substituents at the 7-position on the B-ring largely affect the Qx bands and less the Qy bands. Since the present 7-vinyl group is directly conjugated to the chlorin π -system, the π -elongation along the x-axis as in **1a** \rightarrow **1b** red-shifted the Qx bands. A vinyl group is

withdrawing effect of the 7-vinyl group in **1b** slightly blue-shifted the Qy bands: group electronegativities (χ s) of the methyl and vinyl groups are calculated to be 2.47 and 2.79, respectively.²⁶ The blue shifts were consistent with the observation that a more strongly electron-pulling formyl group at the 7-position of Chl-*b* and its synthetic analogs moved their Qy maxima to shorter wavelengths than those of the corresponding 7-methylated compounds including Chl-*a*.^{3,5,27} The Soret band is produced by a combination of the Bx with By bands, so the Soret maxima of **1b** were bathochromically shifted by a large red-shifted Bx band based on an increase of the π -conjugation along the x-axis rather than a



Scheme 2. Synthesis of zinc methyl pyropheophorbides-*a* Zn-**2a**–**c**: (ix) Zn(OAc)₂·2H₂O/MeOH, CH₂Cl₂.



Fig. 2. Visible absorption (A) and CD spectra (B) of methyl mesopyropheophorbides $1a{-}c$ in THF (ca. 10 μM). All the absorption spectra were normalized at the Soret maxima.

slightly blue-shifted By band proposed from the electronegativity of the 7-vinyl group.

Dehydrogenation of the 8-ethyl group of **1a** to the 8-vinyl group of **1c** did not change the Qy maxima in THF but red-shifted the Qx and Soret maxima. The bathochromic shifts are ascribable to the aforementioned π -conjugation along the x-axis. No shifts observed in the Qy maxima were different from the slight blue shifts by modification of **1a** to **1b**. Since ethyl and methyl groups have almost the same electronegativity [χ (Et) = 2.48 and χ (Me) = 2.47],²⁶ the small difference is due to the substitution effect at the 7- and 8-

Table 1 Visible absorption maxima (nm) of methyl pyropheophorbides **1a-c**, **2a-c**, and Zn-**2a-c** in THF (ca. 10⁻⁵ M).^a

Compound	Soret	Qx		Qy	
		(0,1)	(0,0)	(0,1)	(0,0)
1a	407	502 (9)	533 (9)	601 (7)	657 (44)
1b	417	507 (9)	539 (5)	600 (6)	656 (34)
1c	416	507 (8)	539 (5)	601 (6)	657 (39)
2a	412	508 (10)	537 (8)	610(7)	668 (46)
2b	421	512 (11)	541 (6)	609(7)	666 (36)
2c	421	513 (10)	542 (5)	610 (6)	668 (36)
Zn- 2a	427	526 (4)	568 (6)	609 (11)	656 (67)
Zn- 2b	436	531 (4)	_b	606 (9)	653 (60)
Zn- 2c	436	532 (4)	_ ^b	609 (9)	655 (61)

 $^{\rm a}$ The values in parentheses indicate the absorption intensities (%) relative to the Soret maximum.

^b The band was so broad that the maximum could not be clearly confirmed.

positions to the transition moment along the y-axis. It is noted that the absorption spectrum of **1c** looks like that of **1b** and the differences in their maxima are at most 1 nm, thus a vinyl group on the B-ring affects the visible spectra in a similar manner. The Qy peak positions of **1a**–**c** did not change so dramatically, but the intensity of the Qy(0,0) band relative to that of the redmost Soret peak apparently decreased in the order of **1a** > **1c** > **1b**. Furthermore, substitution effects on the Qy/Soret maxima and relative Qy intensities were confirmed by molecular model calculations (Table S1).

Since Chl-*a*/*b* derivatives **1a**–**c** are chiral and have the same stereochemistry at the 17- and 18-positions, (175,185), circular dichroism (CD) bands were observed in the visible region (Fig. 2B). Negative CD peaks were seen at the Qy(0,0)/(0,1) positions and positive peaks were visible at the Qx(0,0) and Soret maxima. The CD peak intensity at the Qx(0,0) is dependent on the visible absorption intensity.

Excitation of a diluted THF solution of **1a**–**c** at the Soret maxima gave fluorescence emission bands at around 660 nm (Fig. 3B). The slight shifts of their emission peak positions (λ_{em}) were consistent with those of the Qy(0,0) absorption peak positions (λ_{abs}), as expected (Fig. 3 and Table 2). The Stokes shifts (Δ s) of **1a**–**c** were the same and small at 3 nm, indicating that the molecular structures of their ground states were similar to those of their first singlet excited states. Their main emission bands resembled the Qy(0,0) absorption bands in a mirror image. The bandwidths of the former were comparable to those of the latter, and the full widths at half maxima (FWHMs) of the fluorescence bands were only about 10% larger than those of the Qy(0,0) bands. The fluorescence emission quantum yield (Φ_{em}) of **1a** was larger than those of **1b** and **1c**. The vinyl group at the B-ring partly suppressed the Φ_{em} values. The decrease order of **1a** > **1c** > **1b** is interestingly consistent with the order of their relative Qy(0,0) intensities (vide supra). The fluorescence emission of **1a**–**c** decayed in a single exponential manner and their lifetimes (τ_{em} s) were almost the same, hardly varying by 7,8-vinylation.

2.3. Optical properties of methyl pyropheophorbides 2a-c

3-Vinyl-chlorin **2a** in THF showed a similar visible spectral shape as that of 3-ethyl-chlorin **1a** but the dehydrogenation shifted all the peak positions to longer wavelengths (Figs. 2A and 4A). Red shifts of the Qy maxima were about 10 nm and larger than those of the Qx and Soret maxima (ca. 5 nm) (Table 1). The 3-vinyl group elongated the π -conjugation with the chlorin moiety along the y-axis and largely affected the Qy bands, which was consistent with the substitution effect at the 3-position of chlorophyll pigments previously reported.³ Similar shifts were observed in the 3¹,3²-dehydrogenation of **1b/c** to **2b/c**. Close inspection indicated that the Qy(0,0) bands of **2a–c** were broader than those of **1a–c**. The substitution of the 3-ethyl with 3-vinyl group increased FWHMs by about 30% (Table 2). The broadening can be ascribable to the rotational conformation of the 3-vinyl group around the C3–C3¹ single bond.²³

In 3-vinyl-chlorins **2a**–**c**, introduction of a vinyl group at the 7or 8-position induced the same effects in their visible spectra as in 3-ethyl-chlorins **1a**–**c**. Typically, small blue or no shifts of the Qy maxima were observed for the 7- or 8-vinylation, respectively, although it apparently red-shifted the Qx and Soret maxima. All the three 3-vinyl-chlorins **2a**–**c** gave negative CD peaks in the Qy(0,0) regions and positive CD peaks in the Qx(0,0) and redmost Soret regions (Fig. 4B), which was comparable to the CD spectra of 3ethyl-chlorins **1a**–**c**. The 3-vinylation as in **1a**–**c** → **2a**–**c** did not disturb the substitution effect of the 7,8-vinylation on the visible and CD spectra.

By the 3-vinylation of 1a-c to 2a-c, the main fluorescence



Fig. 3. Visible Qy(0,0) absorption (A) and fluorescence emission spectra (B) of methyl mesopyropheophorbides **1a**–**c** in aerated THF. All the spectra were normalized at their most intense peaks and the emission spectra were measured by excitation at the Soret maxima.

Table 2

Qy absorption and fluorescence emission data of methyl pyropheophorbides **1a**–**c**, **2a**–**c**, and Zn-**2a**–**c** in aerated THF at room temperature.^a

Compound	λ_{abs}/nm	λ_{em}/nm	Δ/nm	$\Phi_{\rm em}/\%$	τ_{em}/ns
1a	657 (327)	660 (355)	3	25	6.4
1b	656 (351)	659 (375)	3	20	6.5
1c	657 (299)	660 (325)	3	22	6.5
2a	668 (408)	674 (416)	6	27	6.8
2b	666 (433)	671 (421)	5	19	6.6
2c	668 (401)	673 (403)	5	21	6.6
Zn- 2a	656 (412)	661 (427)	5	21	3.0
Zn- 2b	653 (407)	658 (417)	5	17	3.0
Zn- 2c	655 (392)	660 (414)	5	17	2.9

^a λ_{abs} , Qy(0,0) absorption maximum; λ_{em} , emission maximum (excited at Soret maxima); Δ , Stokes shift = $\lambda_{em} - \lambda_{abs}$; Φ_{em} , emission quantum yield (excited at Soret maxima); τ_{em} , emission lifetime (excited at 403 nm); the values in parentheses indicate full widths at half maxima of bands (cm⁻¹).



Fig. 4. Visible absorption (A) and CD spectra (B) of methyl pyropheophorbides 2a-c in THF (ca. 10 μ M). All the absorption spectra were normalized at the Soret maxima.

emission maxima moved to longer wavelengths (12–14 nm) and their bands widened (50–80 cm⁻¹ for FWHM) (Fig. 5 and Table 2). The Stokes shifts of **2a–c** were 5–6 nm and two times larger than those of **1a–c**. Enhancement of the emission bandwidths and Stokes shifts may be due to the fact that **2a–c** could take various rotamers with different π -conjugations of the 3-vinyl group with the chlorin moiety (vide supra). The 3-vinylation slightly affected the emission quantum yields and single-exponentially obeyed lifetimes (Table 2).

The fluorescence emission quantum yields were reduced in the order of **2a** > **2c** > **2b** and their lifetimes were almost the same. The substitution effects at the 7/8-vinylation of **2a** to **2b/c** were consistent with those of **1a** to **1b/c**. An increase in the absorbance ratio of the Qy(0,0) over Soret maxima enhanced the Φ_{em} values as described in **1a–c**. The linear correlation of the relative Qy(0,0) intensity with Φ_{em} in **1/2a–c** (Fig. S1) is in good agreement with the

observation for natural Chls previously reported.²⁷ Synthetic Chl derivatives bearing a relatively more intense Qy band were more fluorescent.

2.4. Physical properties of zinc methyl pyropheophorbides Zn-2a-c

Zinc metalation of free bases $2\mathbf{a}-\mathbf{c}$ to $Zn-2\mathbf{a}-\mathbf{c}$ induced blue shifts and relative enhancement in intensity of the Qy bands, red shifts and relative reduction of the Qx bands and red shift of Soret maxima (15 nm) in THF (Figs. 4/6A and Table 1). The 7/8-vinylation of Zn-2a to Zn-2b/c affected the visible spectra similarly to those of 1a to 1b/c and 2a to 2b/c: typically, small blue shifts of the Qy(0,0) bands and large red shifts of the Soret maxima. The Qy(0,0) bandwidths of Zn-2a-c were not largely dependent on the 7,8substituents and their band shapes were similar to each other. In Zn-2a-c, negative, faint positive, and intense positive CD peaks were observed in the Qy(0,0), Qx(0,0), and redmost Soret absorption regions, respectively (Fig. 6B). It is noteworthy that the zinc complexes are axially ligated with a THF molecule and the resulting five-coordinated species led to be monomeric in diluted THF solution.

Naturally occurring Chl-*a* was dissolved in diethyl ether to form its 5-coordinated species with a diethyl ether molecule as a single axial ligand.²⁸ Based on the reported data in diethyl ether, the visible absorption maxima of Chl-*a* possessing the 3-vinyl group were compared with those of synthetic 7V-Chl-*a* bearing the 3,7-divinyl groups and natural DV-Chl-*a* carrying the 3,8-divinyl groups (Table 3). The three Qy(0,0) maxima are almost the same at around 660 nm, but the Soret bands of 7V- and DV-Chl-*a* were red-shifted by ca. 5 nm. The reported tendency is comparable to the present observation in Zn-**2a**–**c**. Moreover, the relative Qy(0,0) intensities to the Soret maxima decreased in the order of Chl-*a* > DV-Chl-*a* > 7V-Chl-*a*, which is consistent with that for Zn-**2a**–**c**. Therefore, Zn-**2a**–**c** are good models for the above Chls-*a*.

Zinc complexes Zn-**2a**–**c** in THF were fluorescent with the same Stokes shifts of 5 nm (Fig. 7 and Table 2). Zinc metalation of **2a**–**c** to Zn-**2a**–**c** decreased the fluorescence emission quantum yields and lifetimes, which are ascribable to the heavy atom effect of the central zinc. The 7/8-vinylation of Zn-**2a** to Zn-**2b**/**c** reduced the quantum yields, which were dependent on the relative Qy(0,0) intensities.

The first oxidation potentials $(E_{1/2}^{ox})$ of Zn-2a-c in THF were



Fig. 5. Visible Qy(0,0) absorption (A) and fluorescence emission spectra (B) of methyl pyropheophorbides $2\mathbf{a}-\mathbf{c}$ in aerated THF. All the spectra were normalized at their most intense peaks and the emission spectra were measured by excitation at the Soret maxima.

determined by cyclic voltammetry: $E_{1/2}^{0x}(Zn-2a) = 340 \text{ mV}$ and $E_{1/2}^{0x}(Zn-2b) = E_{1/2}^{0x}(Zn-2c) = 360 \text{ mV}$ (*vs.* Ag/Ag⁺). Substitution of a methyl or ethyl group on the B-ring with a vinyl group increased the $E_{1/2}^{0x}$ value by 20 mV, irrespective of the introduced position. Since a vinyl group is more electron-withdrawing than alkyl groups (vide supra), and the chlorin π -system directly conjugated with a vinyl group was less electrochemically oxidized. The substitution effect was confirmed by the observation that Zn-1a possessing the 3-ethyl group was more oxidized by 20 mV than the 3-vinyl analog Zn-2a: $E_{1/2}^{0x}(Zn-1a) = 320 \text{ mV}$. The change of $E_{1/2}^{0x}$ by substitution with a vinyl group was similar to the reported data of $E_{1/2}^{0x}$ (DV-Chl-a) – $E_{1/2}^{0x}$ (Chl-a) = ca. 10 mV.⁸

The above results provide the following effects by the 7,8-vinylation. The intense Soret band moves to a longer wavelength by the 7,8-vinylation and more largely overlaps with the solar spectrum so the pigments can absorb sunlight more efficiently. The 7,8-vinylation affects the Qy(0,0) band only slightly so that the energy-donating and accepting abilities of the Chl molecules and their singlet excited energies are not so largely changed. Additionally, the oxidation potentials faintly increase by the 7,8-vinylation and the electron transfer processes are primarily independent of the substitution. In the initial stages of photosynthesis, the divinylated Chls are more useful for absorbing and harvesting sunlight than monovinylated Chl-*a*, and are also comparable in excited energy and electron transferring events to Chl-*a*.

3. Concluding remarks

The present synthetic Chl derivatives showed characteristic optical and electrochemical properties in THF which were dependent on the peripheral substituents. The substitution of an alkyl group with a vinyl group on the B-ring shifted the Soret and Qx bands bathochromically and induced no or small blue shifts of the



Fig. 6. Visible absorption (A) and CD spectra (B) of zinc methyl pyropheophorbides Zn-**2a**–**c** in THF (ca. 10 μ M). All the absorption spectra were normalized at the Soret maxima.

 Table 3

 Visible absorption maxima (nm) of chlorophylls in diethyl ether reported previously.

Compound	Soret	Qy(0,0) ^a	Reference
Chl-a	432	662 (76)	19
	430	660 (80)	29
	430	661 (77)	3
7V-Chl- <i>a</i> ^b	437	660 (65)	19
DV-Chl-a	435 436	660 (71) 660 (71)	29 3

 $^{\rm a}$ The values in parentheses indicate the absorption intensities (%) relative to the Soret maximum.

^b 7-Demethyl-7-vinyl-chlorophyll-a.

Qy maxima. The vinylation partly decreased the relative Qy(0,0) peaks to the Soret maxima and fluorescence emission quantum yields, but the emission lifetimes were independent of the modification. These substitution effects are ascribable to a π -conjugatable and electron-withdrawing vinyl group along the molecular x-axis. The substitution position of a vinyl group on the B-ring hardly affected the optical properties, although Qy(0,0) absorption and fluorescence emission maxima of 7-vinyl-chlorins were shifted more hypsochromically by 1–2 nm than those of the corresponding 8-vinyl-chlorins. The oxidation potentials slightly increased by the vinylation due to the electron-withdrawing vinyl group, in which no substitution position effect was observed.

Synthetic 7-vinylated Chl-*b* derivatives exhibited similar optical and electrochemical properties as those of the 8-vinylated Chl-*a* derivatives. Similarly to DV-Chl-*a*, 7V-Chl-*a* would be useful for a photosynthetic pigment but has never been found in any phototrophs. The unavailability is due to its biosynthetic inconvenience including the complicated transformation of the 7-methyl to vinyl group. In the biosynthesis of Chl-*a*, the 8-vinyl group of its precursor is hydrogenated to the 8-ethyl group, so the reductase is inactivated to readily produce DV-Chl-*a*.¹²

The vinyl groups on the B-ring are coplanar, with the chlorin π system taking an energetically stable conformation in solution since they can be freely rotated around the C7–C7¹ or C8–C8¹ single bond. In photosynthetic apparatuses, the vinyl groups are fairly fixed by specific interaction of environmental peptides and their rotation from coplanar to perpendicular conformation



Fig. 7. Visible Qy(0,0) absorption (A) and fluorescence emission spectra (B) of zinc methyl pyropheophorbides Zn-**2a**–**c** in aerated THF. All the spectra were normalized at their most intense peaks and the emission spectra were measured by excitation at the Soret maxima.

4. Experimental

4.1. General

All melting points were measured with a Yanagimoto micro melting apparatus and were uncorrected. Visible absorption and CD spectra were measured with a Hitachi U-3500 spectrophotometer and a Jasco J-720W spectropolarimeter, respectively. The fluorescence emission spectra and guantum yields were obtained by a Hamamatsu Photonics C9920-03G spectrometer. Fluorescence emission lifetimes were determined by a Hamamatsu Photonics C7990S system. Cyclic voltammetry was performed by a BAS CV-50W analyzer with a conventional three-electrode system (platinum working/counter electrodes and aq. 0.1 M silver nitrate for reference): a THF solution of tetraethylammonium and tetrabutylammonium perchlorates (each 0.1 M) and scan rate = 2000 mV/s. FT-IR spectra were recorded on a Shimadzu IRAffinity-1 spectrophotometer and a Shimadzu AIM-8000R microscope was used for measurements of solid films on an aluminum coated glass (reflection mode). ¹H and ¹³C NMR spectra were recorded on a IEOL ECA-600 (600 and 151 MHz) spectrometer: CHCl₃ ($\delta = 7.26$ ppm) and ¹³CDCl₃ ($\delta = 77.0$ ppm) were used as internal references. Standard mass data were obtained using laser desorption/ionization (LDI) by a Shimadzu AXIMA-CFR plus spectrometer. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF II spectrometer: atmospheric pressure chemical ionization (APCI) and positive mode in a methanol solution. TLC or flash column chromatography (FCC) was performed with silica gel (Merck, Kieselgel 60 F₂₅₄ or Kieselgel 60, 40–63 μm, 230–400 mesh).

All the reactions were carried out in the dark under nitrogen. Methyl mesopyropheophorbide-*a* (**1a**),¹⁴ methyl 7-demethyl-7-vinyl-mesopyropheophorbide-*a* (**1b**),⁵ methyl 8-deethyl-8-vinyl-mesopyropheophorbide-*a* (**1c**),⁵ methyl pyropheophorbide-*a* (**2a**),⁵ methyl *cis*-7,8-dihydroxy-bacteriopheophorbide-*a* (**2d**),¹⁵ and methyl 7¹-hydroxy-pyropheophorbide-*a* (**2f**)⁵ were prepared according to reported procedures. Solvents and reagents for preparation of the compounds were obtained from commercial suppliers and utilized as supplied. THF for optical spectroscopy was purchased from Nacalai Tesque as reagent prepared specially for high performance liquid chromatography and used without further purification.

4.2. Synthesis of methyl pyropheophorbides

4.2.1. Synthesis of methyl pyropheophorbide-b (2e)

A dry dichloromethane solution (30 ml) of primary alcohol **2f** (47.6 mg, 84.4 μ mol) was treated with an excess amount of PDC (200 mg, 532 μ mol) and stirred at room temperature for 5 h. After disappearance of **2f** on a TLC plate, the reaction mixture was filtered on Celite and the solvent was evaporated. The residue was purified by recrystallization (CH₂Cl₂ and hexane) to give aldehyde **2e** (26.0 mg, 46.3 μ mol, 55%): see the spectral data in Ref. 31.

4.2.2. Synthesis of methyl 7-demethyl-7-vinyl-pyropheophorbide-a (**2b**)

Potassium *tert*-butoxide (84.5 mg, 753 μ mol) and methyltriphenylphosphonium iodide (310 mg, 763 μ mol) were added to dry dichloromethane (12 ml) and stirred at room temperature under argon for 1 min. After the mixture had turned to a

brilliant vellow color, a dry dichloromethane solution (1 ml) of aldehyde **2e** (10.0 mg, 17.8 µmol) was added and stirred for 1 min. The reaction mixture was guenched with water and extracted with dichloromethane several times. The combined dichloromethane phases were washed with water, dried over sodium sulfate, and filtered. After the solvent was evaporated, the residue was purified by FCC (3% Et₂O and CH₂Cl₂) and recrystallization (CH₂Cl₂ and hexane) to give olefin **2b** (4.2 mg, 7.5 µmol, 42%): black solid; mp 104–105.5 °C; VIS (CH₂Cl₂) λ_{max} 664 (relative intensity, 0.16), 597 (0.07), 569 (0.07), 521 (0.08), 425 nm (1.00); IR (film) v_{max} 1737 $(17^2-C=0)$, 1692 cm⁻¹ (13-C=0); ¹H NMR (CDCl₃) δ 9.59 (1H, s, 10-H), 9.58 (1H, s, 5-H), 8.57 (1H, s, 20-H), 7.99 (1H, dd, J = 18, 12 Hz, 3-CH), 7.89 (1H, dd, *J* = 18, 12 Hz, 7-CH), 6.29 (1H, dd, *J* = 18, 1 Hz, 3¹-CH trans to 3-C–H), 6.17 (1H, dd, J = 12, 1 Hz, 3¹-CH cis to 3-C–H), 6.13 (1H, dd, *J* = 18, 1 Hz, 7¹-CH *trans* to 7-C–H), 5.98 (1H, dd, *J* = 12, 1 Hz, 7^1 -CH *cis* to 7-C–H), 5.28, 5.13 (each 1H, d, J = 19 Hz, 13^1 -CH₂), 4.49 (1H, dq, J = 2, 7 Hz, 18-H), 4.30 (1H, dt, J = 8, 2 Hz, 17-H), 3.83 $(2H, q, J = 8 Hz, 8-CH_2)$, 3.68 $(3H, s, 12-CH_3)$, 3.61 $(3H, s, 17^2-$ COOCH₃), 3.41 (3H, s, 2-CH₃), 2.71 (1H, m, 17-CH), 2.57 (1H, m, 17¹-CH), 2.30 (2H, m, 17-CHCH), 1.82 (3H, d, J = 7 Hz, 18-CH₃), 1.76 (3H, t, J = 8 Hz, 8^{1} -CH₃), 0.36, -1.71 (each, 1H, s, NH x 2); MS (APCI) found: *m*/*z* 561.2048. Calcd. for C₃₅H₃₇N₄O₃: MH⁺, 561.2060.

4.2.3. Synthesis of methyl 8-deethyl-8-vinyl-pyropheophorbide-a (2c)

p-Toluenesulfonic acid monohydrate (138.3 mg, 727 umol) was added to a THF solution (40 ml) of triol 2d (222.2 mg, 370 µmol). The mixture was refluxed for 1 h and cooled down to room temperature. The reaction mixture was poured into an aqueous 4% sodium bicarbonate solution and extracted with dichloromethane several times. The combined dichloromethane phases were washed with water, dried over sodium sulfate, and filtered. After the solvents were evaporated, the residue was dissolved in 1,2dichlorobenzene (50 ml) and refluxed for 5 h. The solvent was removed in vacuo and the residue was purified by FCC (3–5% Et₂O and CH₂Cl₂) and recrystallization (CH₂Cl₂ and hexane) to give olefin 2c (66.7 mg, 122 µmol, 33%): dark green solid; mp 194–195 °C; VIS $(CH_2Cl_2) \lambda_{max}$ 667 (relative intensity, 0.37), 610 (0.07), 543 (0.05), 513 (0.09), 422 (1.00), 404 (shoulder, 0.71), 322 nm (18); IR (film) ν_{max} 1737 (17²-C=O), 1695 cm⁻¹ (13-C=O); ¹H NMR (CDCl₃) δ 9.66 (1H, s, 5-H), 9.45 (1H, s, 10-H), 8.58 (1H, s, 20-H), 8.02 (1H, dd, *J* = 18, 12 Hz, 8-CH), 7.92 (1H, dd, *J* = 18, 11 Hz, 3-CH), 6.30 (1H, dd, J = 18, 1 Hz, 8^{1} -CH trans to 8-C–H), 6.19 (1H, dd, J = 12, 1 Hz, 8^{1} -CH *cis* to 8-C–H), 6.15 (1H, dd, *J* = 18, 1 Hz, 3¹-CH *trans* to 3-C–H), 6.00 (1H, dd, J = 11, 1 Hz, 3¹-CH *cis* to 3-C-H), 5.27, 5.12 (each 1H, d, J = 19 Hz, 13^{1} -CH₂), 4.50 (1H, dq, J = 2, 8 Hz, 18-H), 4.30 (1H, dt, J = 7, 2 Hz, 17-H), 3.66 (3H, s, 7-CH₃), 3.62 (3H, s, 17²-COOCH₃), 3.42 (3H, s, 2-CH₃), 3.36 (3H, s, 12-CH₃) 2.74-2.69, 2.61-2.55 (each 1H, m, 17¹-CH₂), 2.34–2.28 (2H, m, 17-CH₂), 1.82 (3H, d, *J* = 8 Hz, 18-CH₃), 0.33, -1.75 (each 1H, s, NH x 2); 13 C NMR (CDCl₃) δ 196.3, 173.7, 171.7, 160.9, 154.2, 149.8, 149.1, 141.8, 139.2, 138.0, 137.2, 136.1, 136.0, 131.7, 130.7, 130.1, 129.3, 129.2, 122.8, 119.8, 106.1, 105.2, 97.5, 93.2, 51.9, 51.9, 50.1, 48.2, 31.2, 30.0, 23.4, 12.5, 12.3, 12.1; MS (LDI) found: m/z 547. Calcd. for C₃₄H₃₅N₄O₃: MH⁺, 547; HRMS (APCI) found: m/z547.2704. Calcd. for C₃₄H₃₅N₄O₃: MH⁺, 547.2704: see also the spectral data in Ref.¹⁷

Alternatively, treatment of triol **2d** with *p*-toluenesulfonic acid monohydrate in refluxing dichloromethane and benzene (1:9) for 3 h¹⁸ afforded **2c** in a yield of 9%.

In addition, the Wittig reaction of methyl 8-vinyl-pyropheophorbide- d^{15} with methylenetriphenylphosphorane (section 4.2.2) gave **2c** (46%).

4.3. Synthesis of zinc methyl pyropheophorbides-a

4.3.1. Zinc metalation

A methanol solution saturated with zinc acetate dihydrate (1 ml) was added to a dichloromethane solution (5 ml) of free bases **1a** and **2a**–**c** (2 μ mol) and the mixture was stirred at room temperature for 30 min. The reaction mixture was washed with an aqueous 4% sodium bicarbonate solution and water, dried over sodium sulfate, and filtered. After the solvents were evaporated, the residue was reprecipitated from dichloromethane and hexane to give the corresponding zinc complexes Zn-**1a** and Zn-**2a**–**c** nearly quantitatively.

4.3.2. Spectral data of zinc complexes Zn-1a/2a-c

Zinc methyl mesopyropheophorbide-*a* (Zn-**1a**): VIS (THF) λ_{max} 644 (relative intensity, 0.63), 602 (0.10), 565 (0.06), 520 (0.04), 423 (1.00), 403 nm (0.59); MS (LDI) found: *m/z* 612.4. Calcd. for C₃₄H₃₆N₄O₃Zn: M⁺, 612.2: see also the spectral data in Refs.^{32–34}

Zinc methyl pyropheophorbide-*a* (Zn-**2a**): VIS (THF) λ_{max} 656 (relative intensity, 0.67), 609 (0.11), 568 (0.06), 526 (0.04), 427 (1.00), 406 (0.55), 380 nm (0.33); MS (LDI) found: *m/z* 610.1. Calcd. for C₃₄H₃₄N₄O₃Zn: M⁺, 610.2: see also the spectral data in Refs.^{32,35,36}

Zinc methyl 7-demethyl-7-vinyl-pyropheophorbide-*a* (Zn-**2b**): VIS (THF) λ_{max} 653 (relative intensity, 0.60), 606 (0.09), 531 (0.04), 436 (1.00), 378 nm (0.24); MS (LDI) found: *m*/*z* 622.1. Calcd. for C₃₅H₃₄N₄O₃Zn: M⁺, 622.2.

Zinc methyl 8-deethyl-8-vinyl-pyropheophorbide-*a* (Zn-**2c**): VIS (THF) λ_{max} 655 (relative intensity, 0.61), 609 (0.09), 532 (0.04), 436 (1.00), 380 nm (0.25); MS (LDI) found: *m*/*z* 608.3. Calcd. for C₃₄H₃₂N₄O₃Zn: M⁺, 608.2.

Acknowledgements

We would like to thank Dr. Meiyun Xu of Ritsumeikan University for her experimental assistance. This work was partially supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Artificial Photosynthesis (AnApple)" (No. 24107002) from the Japan Society for the Promotion of Science (JSPS).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2016.12.003.

References

- Blankenship RE. Molecular Mechanisms of Photosynthesis. second ed. Hoboken, NJ: Wiley-Blackwell; 2014. Chap. 4, pp. 41–58.
- 2. Croce R, van Amerongen H. Nat Chem Biol. 2014;10:492.
- 3. Tamiaki H, Kunieda M. Handbook of Porphyrin Science. vol. 11. 2011:223-290.
- 4. Chen M. Annu Rev Biochem. 2014;83:317.
- 5. Tamiaki H, Tsuji K, Kim K, Miyatake T. Tetrahedron. 2016;72:4368.
- 6. Ting CS, Rocap G, King J, Chisholm SW. Trends Microbiol. 2002;10:134.
- 7. Ito H, Tanaka A. Proc Natl Acad Sci USA. 2011;108:18014.
- 8. Komatsu H, Wada K, Kanjoh T, et al. Photosynth Res. 2016;130:445.
- 9. Mizoguchi T, Nagai C, Kunieda M, Kimura Y, Okamura A, Tamiaki H. Org Biomol Chem. 2009;7:2120.
- 10. Álvarez S, Zapata M, Garrido JL, Vaz B. Chem Commun. 2012;48:5500.
- 11. Goericke R, Repeta DJ. Limnol Oceanogr. 1992;37:425.
- 12. Tamiaki H, Teramura M, Tsukatani Y. Bull Chem Soc Jpn. 2016;89:161.
- Tamiaki H, Machida S, Mizutani K. J Org Chem. 2012;77:4751.
 Tamiaki H, Ariki N, Sugiyama H, Taira Y, Kinoshita Y, Miyatake T. Tetrahedron. 2013;69:8412.
- 15. Kunieda M, Mizoguchi T, Tamiaki H. Tetrahedron. 2004;60:11349.
- 16. Tamiaki H, Omoda M, Saga Y, Morishita H. Tetrahedron. 2003;59:4337.
- 17. Zheng G, Dougherty TJ, Pandey RK. J Org Chem. 1999;64:3751.
- 18. Xu M, Kinoshita Y, Matsubara S, Tamiaki H. Photosynth Res. 2016;127:335.
- 19. Michalski TJ, Hunt JE, Hindman JC, Katz JJ. Tetrahedron Lett. 1985;26:4875.
- 20. Fischer R, Engel N, Henseler A, Gossauer A. Helv Chim Acta. 1994;77:1046.

- Gerlach B, Brantley SE, Smith KM. J Org Chem. 1998;63:2314.
 Wang L-M, Wang Z, Yang Z, Jin Y-X, Wang J-J. Chin J Org Chem. 2012;32:2154. Tamiaki H, Tsuji K, Kuno M, Kimura Y, Watanabe H, Miyatake T. Bioorg Med 23. Chem Lett. 2016;26:3034.
- Ito H, Yokono M, Tanaka R, Tanaka A. J Biol Chem. 2008;283:9002.
 Tamiaki H, Takekoshi D, Mizoguchi T. Bioorg Med Chem. 2011;19:52.
- 26. Inamoto N, Masuda S. Chem Lett. 1982;11:1003.
- 27. Kobayashi M, Sorimachi Y, Fukayama D, et al. In: Pessarakli M, ed. Handbook of
- *Photosynthesis*. third ed. Boca Raton, FL: CRC press; 2016. chap. 6, pp. 95–147. 28. Fujiwara M, Tasumi M. J Phys Chem. 1986;90:250.
- 29. Shedbalkar VP, Rebeiz CA. Anal Biochem. 1992;207:261.
- 30. Tomo T, Akimoto S, Ito H, et al. Biochim Biophys Acta. 2009;1787:191.
- Tambi A, Hallido S, Ho H, et al. *Diction Diopys Acta*. 2
 Tambi A, J Kundo M, Oba T. *Tetrahedron*. 2000;56:6245.
 Simpson DJ, Smith KM. *J Am Chem Soc*. 1988;110:2854.
- 33. Tamiaki H, Amakawa M, Shimono Y, Tanikaga R, Holzwarth AR, Schaffner K. Photochem Photobiol. 1996;63:92.
- Tamiaki H, Yagai S, Miyatake T. Bioorg Med Chem. 1998;6:2171.
 Tamiaki H, Watanabe T, Miyatake T. J Porphyrins Phthalocyanines. 1999;3:45.
- 36. Katterle M, Holzwarth AR, Jesorka A. Eur J Org Chem. 2006:414.