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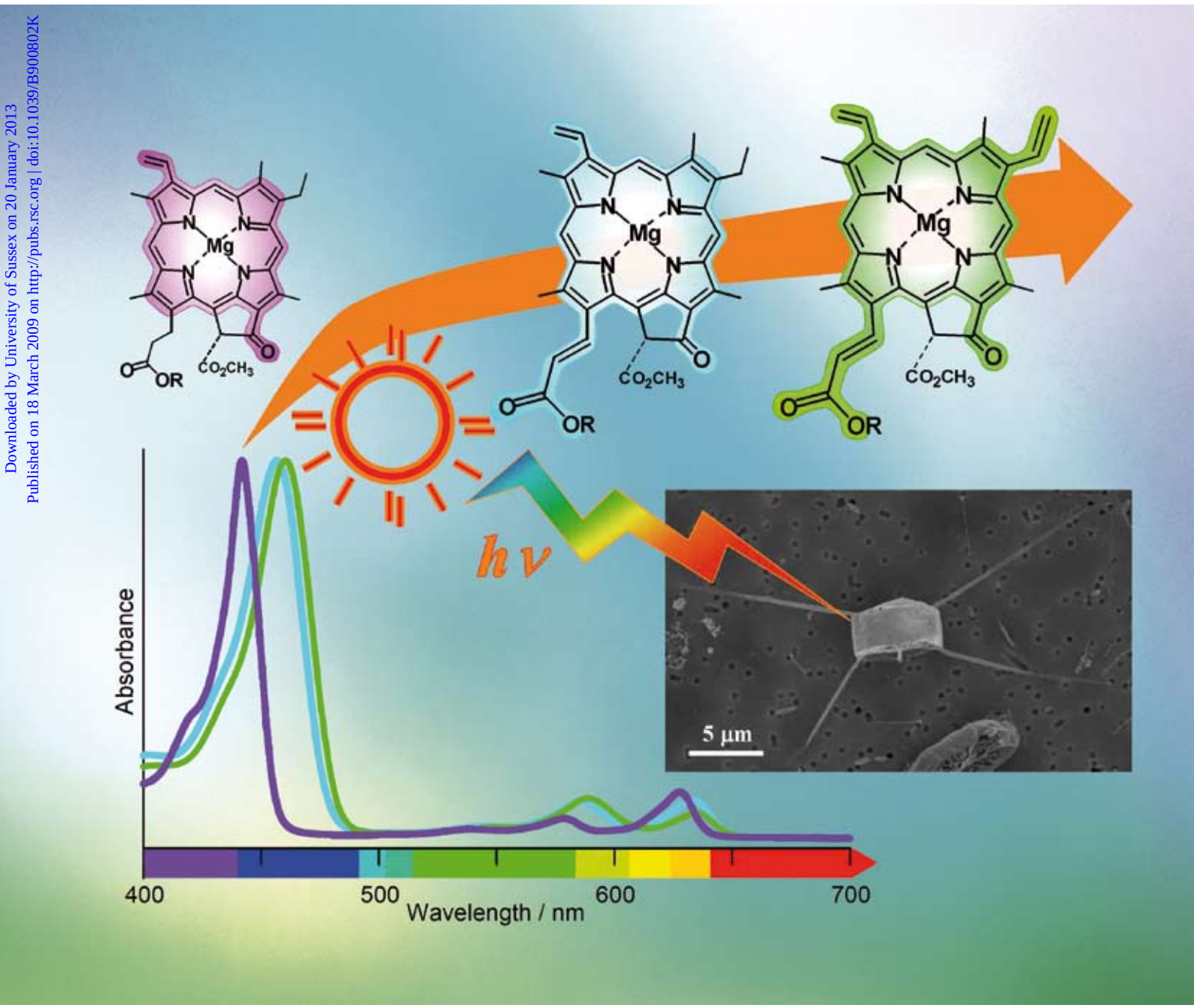

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Stereochemical determination of the unique acrylate moiety at the 17-position in chlorophylls-*c* from a diatom *Chaetoseros calcitrans* and its effect upon electronic absorption properties†

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Chlorophyll (Chl)-c_1 and Chl-c_2 were extracted from a commercially available diatom *Chaetoseros calcitrans*, and the former (8-ethyl) and the latter (8-vinyl) were efficiently separated by reverse-phase HPLC using a polymeric octadecylsilyl column to afford analytically pure compounds in an amount adequate for further chemical modification. The conformation of the unique acrylate moiety at the 17-position of isolated Chls-*c* in THF was unambiguously determined to be "*cisoid*" around the C17–C17^1 bond using ^{1}H-^{1}H NOE correlations: C17^1=C17^2 was on the same side as C17=C18. Interestingly, correlations originating from the "*transoid*" conformer could not be observed under the present NMR conditions, indicating that the rotation of the acrylate was considerably restricted. To elucidate the function of the rigid acrylate in Chls-*c*, we examined their electronic absorption properties using two synthetic types of esters possessing a porphyrin π-system: acrylate-type (17-CH=CH–COOR) prepared by esterification of natural Chl-c_1 and Chl-c_2, and propionate-type (17-CH_2–CH_2–COOR) by 17,18-oxidation of natural Chl-*a* and its 8-vinyl analog. The Soret absorption bands at around 450 nm of the acrylate-type were red-shifted and broadened more than those of the propionate-type. Consequently, the unique acrylate in Chls-*c* serves as an aid for expanding the absorption region around 400–500 nm in order to capture intense irradiation from the sun for photosynthesis.

Introduction

Chlorophylls (Chls)-*c* are widely distributed and abundant in chromophyte algae and some prokaryotes.[1–3] These pigments are bound to proteins in light-harvesting antennae of photosynthetic organisms, and function to absorb sunlight, migrating the excitation energy and transferring it to Chl-*a* molecules, although neither the detailed three-dimensional structure of Chl-*c* containing antenna complexes nor the mechanism of the above photoreactions have been unraveled.[4,5] All Chl-*c* molecules are characterized by a fully conjugated porphyrin π-system as well as a unique acrylate moiety at the 17-position (17-CH=CH–COOR) and most of them lack a long hydrocarbon chain on the acrylate (R = H) (see Fig. 1(a)), whereas usual chlorophyllous pigments have partially saturated chlorin or bacteriochlorin π-systems, propionate residues and long chains as their esters (17-CH_2–CH_2–COOR, R = phytyl *etc.*).[3,6–8]

Recent progress in HPLC methods has led to the discoveries of new Chl-*c*-like pigments and, as a result, eleven types of Chls-*c* have been identified [see ref. 2 and 9 for comprehensive reviews and references cited therein]. Here, we focus our attention on the c_1- and c_2-types of Chl-*c* which are defined by the presence of an 8-ethyl and an 8-vinyl substituent, respectively (see Fig. 1(a)), since the situation is reminiscent of 8-ethylated protochlorophyllide (PChlide)-*a* and its 8-vinyl precursor, found in the biosynthesis of Chl-*a* in oxygenic photosynthetic organisms and bacteriochlorophyll (BChl)-*a* in anoxygenic photosynthetic bacteria.[10]

A major distinction among various types of chlorophyllous pigments is the degree of unsaturation of their conjugated

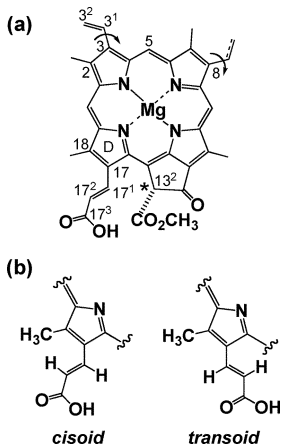

Fig. 1 Molecular structures of Chl-c_1 (8-ethyl) and Chl-c_2 (8-vinyl) (a) and the *cisoid* and *transoid* conformations of the 17-acrylate moiety on the D-ring (b).

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† Electronic supplementary information (ESI) available: Table of red-shift values of absorption maxima induced by esterification at the 17^2-COOH as well as 8^1,8^2- and 17^1,17^2-dehydrogenations of natural Chls-*c* and their analogs; partial HMBC and NOESY spectra of Chl-c_1; total calculated energies of Chl-c_1 and Chl-c_2 as a function of clockwise rotation around the C17–C17^1 bond for the acrylate, C3–C3^1 for the 3-vinyl and C8–C8^1 for the 8-vinyl substituents; ^{1}H NMR spectra of synthetic Chl-c_1-phy, Chl-c_2-phy, PChl-*a* and 8-vinyl-PChl-*a*. See DOI: 10.1039/b900802k

π-systems, which defines their characteristic electronic absorption features.[6,7] The fully conjugated porphyrin π-system as in Chls-*c* and PChlides-*a* is characterized by an intense electronic absorption band in the lower wavelength region (Soret band, ε ≈ 150000) and a moderate band at around 620 nm (Qy band, ε ≈ 20000).[11,12] The exact molecular extinction coefficients (ε) of naturally occurring Chls-*c* have not yet been determined, since large-scale preparation and low solubility in general organic solvents of Chls-*c* are considerably troublesome primarily due to their hydrophilic properties.

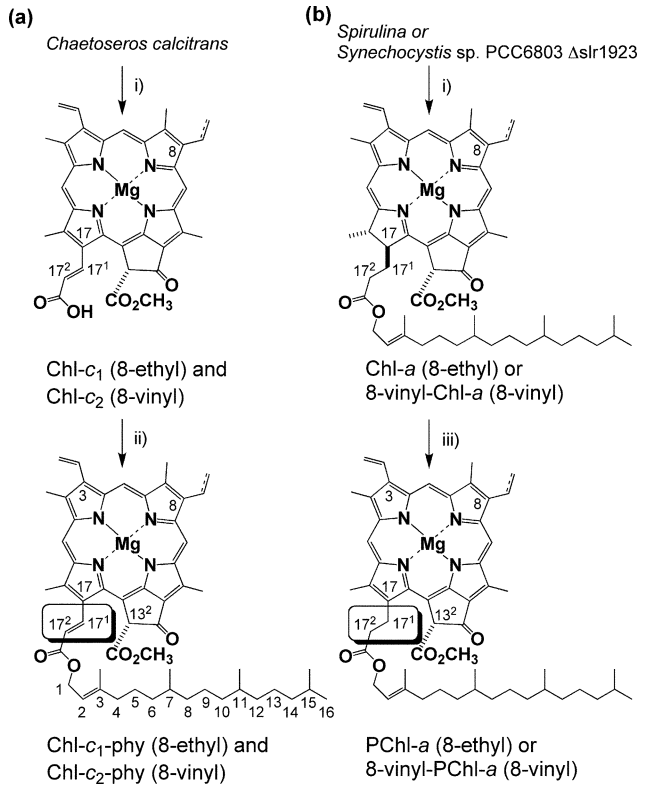
Previous reports on stereochemical characterization of Chls-*c* showed that only the *trans*-orientation of 17^1-H and 17^2-H in the acrylate moiety was unambiguously determined using the vicinal coupling between the two protons in NMR spectroscopy, $^3J_{HH}$ = 16–17 Hz.[13,14] However, these protons were tentatively assigned on the basis of additive properties in their chemical shifts. Therefore, the conformation of the acrylate around the C17–C17^1 bond, *cisoid* and/or *transoid* as shown in Fig. 1(b), has not yet been elucidated. The stereochemistry at the chiral 13^2-position indicated by an asterisk in Fig. 1(a) was demonstrated to be mainly 13^2-(*R*)-configuration using circular dichroism (CD) spectra.[15]

In order to determine the conformation of the π-conjugated acrylate in Chls-*c* and to elucidate its function, it was necessary to prepare a large amount of pure Chls-*c*. In the first part of this paper, we report efficient isolation of Chl-c_1 and Chl-c_2 by reverse-phase HPLC using a polymeric octadecylsilyl (ODS) column and the conformational characterization by ^{1}H- and ^{13}C-NMR spectroscopy. In the second part, we report facile synthesis of Chl-*c* derivatives having a phytyl (C_{20}) substituent at the 17^3-position to enhance their solubility in organic solvents, and their electronic absorption properties in THF. For comparison, we synthesized the corresponding 17^1,17^2-dihydrogenated derivatives having a phytyl propionate-type ester substituent by modification of naturally occurring Chl-*a* and its 8-vinyl analog (see Scheme 1). By means of electronic absorption spectroscopy, we discuss the physiological meaning of the unique acrylate moiety in Chls-*c*.

Results and discussion

Molecular structures of Chl-c_1 and Chl-c_2

Fig. 1(a) depicts the molecular structures of Chl-c_1 and Chl-c_2 investigated here. These Chls-*c* were characterized by the fully conjugated porphyrin π-system as well as the 17-acrylic acid moiety (17-CH=CH–COOH). Chl-c_1 and Chl-c_2 were defined by their peripheral substituents at the 8-position: the 8-ethyl group for Chl-c_1 and the 8-vinyl for Chl-c_2. The *trans*-configuration at the C17^1=C17^2 bond of 17-CH=CH–COOH had already been determined using the vicinal coupling between 17^1-H and 17^2-H in NMR spectroscopy.[13,14] It was consistent with the present observation: $^3J_{HH}$ = 15.8 Hz for Chl-c_1 and 16.0 Hz for Chl-c_2 (see the Experimental section). However, no clear evidence for the stereochemical conformation of the acrylate around the C17–C17^1 bond has yet been available. Thus, two conformations of the acrylate as a pair of rotational isomers were expected as shown in Fig. 1(b) for "*cisoid*"- (left) and "*transoid*"-conformers (right). The stereochemistry at the chiral 13^2-position in Chls-*c*, indicated by an asterisk in Fig. 1(a), was demonstrated to be 13^2-(*R*)-configuration which is generally seen in naturally occurring



Scheme 1 Synthesis of Chls-*c*-phy by esterifying naturally occurring Chls-*c* with a phytyl substituent (a, acrylate type) and PChl-*a* and its 8-vinyl analog by modifying natural Chl-*a* and its 8-vinyl-analog (b, propionate type): i) extraction; ii) phytyl bromide, CsF, DMF; iii) DDQ, acetone.

(B)Chl pigments based on X-ray crystallographic[16,17] and CD spectral analyses.[15] In this study, we used a mixture of the 13^2-enantiomers, since the stereochemistry did not affect the present conformational characterization or the optical absorption spectroscopy of monomeric pigments in solution.

Separation of Chl-c_1 and Chl-c_2 by reverse-phase HPLC using a polymeric ODS

Preparation and separation of a large amount of pure Chl-c_1 and Chl-c_2 were considerably troublesome due to their hydrophilic properties (the presence of a carboxy group in the acrylate). Several HPLC methods for the separation are available.[2,9] However, these reported methods have been mainly used for analytical purposes, not for preparative ones. In this study, we chose an isocratic reverse-phase HPLC method using a polymeric ODS column and a mixture of methanol, acetonitrile and ammonium acetate buffer (pH 5.25) as an eluent, with modification of reported methods.[9,12,18] This enabled us to prepare a large quantity of analytically pure Chl-c_1 and Chl-c_2. Fig. 2(a) depicts a *preparative*-scale HPLC profile for the extracts from the cells of *Chaetoseros calcitrans*. We were able to detect two well-separated peaks as the major components at the retention time of around 13–16 min. Artifacts during handling of materials are indicated by an asterisk in Fig. 2(a). The two peaks are termed peaks #1 and #2 in order of elution. The electronic absorption spectra of each peak were also recorded as shown in Fig. 2(b). In comparison with the spectra, peak #2 indicated by the dotted line exhibited a more red-shifted

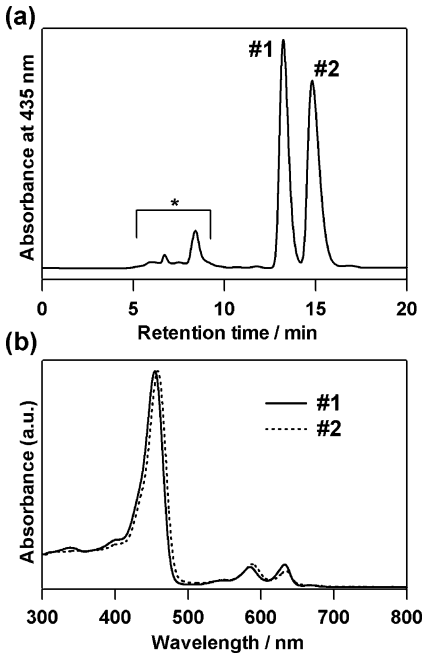


Fig. 2 A preparative-scale HPLC profile for the extracts from a diatom, *Chaetoseros calcitrans* (a) and the absorption spectra of peak #1 (solid) and #2 (dotted) in THF (b). Elution bands bands in the asterisk region corresponded to impurities. See HPLC conditions in the Experimental section. Both spectra were normalized at the Soret maxima.

Soret absorption at 458.4 nm than that of peak #1 (454.6 nm) by the solid line. Based on absorption data of Chls-*c* previously reported,[2,12] peaks #1 and #2 were assigned to be Chl-c_1 having an 8-ethyl substituent and Chl-c_2 having an 8-vinyl substituent, respectively. The assignment was simultaneously confirmed using electron spray ionization (ESI)-LCMS, followed by ^{1}H- and ^{13}C-NMR spectra (see details in the Experimental section). It is noted that the present HPLC method using an *achiral* column could not separate a pair of enantiomeric isomers at the 13^2-position as mentioned above.

Conformational characterization of the 17-acrylate moiety in Chls-*c*

To determine the stereochemical conformation of the 17-acrylate moiety in naturally occurring Chls-*c* as shown in Fig. 1(b), first we tried to obtain a complete set of assignments of ^{1}H signals in the NMR spectra in THF-d_8. All their proton signals were readily assigned using ^{1}H-^{1}H correlation spectroscopy (COSY) and ^{1}H–^{1}H nuclear Overhauser effect spectroscopy (NOESY) spectra, except for 17^1-H and 17^2-H signals which were key protons to identify the conformation of the acrylate based on NOE correlations. These two protons could not be identified using the above ^{1}H-NMR techniques due to the presence of two possible conformers around the C17–C17^1 bond shown in Fig. 1(b). Therefore, a set of assignments of the ^{13}C signals of Chls-*c* would be essential to identify the two protons using ^{1}H-^{13}C correlations starting from the isolated carbonyl carbon signal at the 17^3-position. The assignments of both ^{1}H and ^{13}C signals in Chl-c_1 and Chl-c_2 thus obtained are summarized in the Experimental section. The remaining 17^1-H and 17^2-H signals were unambiguously assigned using heteronuclear multiple-bond correlation (HMBC) spectra (see partial HMBC spectrum for Chl-c_1 in Fig. S1†): δ(17^1-H) = 9.09 and δ(17^2-H) = 6.71 ppm for Chl-c_1 and δ(17^1-H) = 9.10 and δ(17^2-H) = 6.74 ppm for Chl-c_2. These assignments were consistent with those reported already (but ambiguously[13,14]) based on additive properties in their chemical shifts. The *trans*-orientation of 17^1-H and 17^2-H was also confirmed, since no detectable NOE correlations were observed among the two protons besides their vicinal coupling (= 16 Hz) as mentioned above.

After obtaining a complete set of assignments of the proton signals, we carefully re-examined ^{1}H-^{1}H NOESY spectra of both Chl-c_1 and Chl-c_2. The correlations between 17^1-H and 13^2-H and between 17^2-H and 18-CH$_3$ were clearly observed, indicating that the conformation of the 17-acrylate moiety was *cisoid* (see Fig. S2† for the NOESY spectrum of Chl-c_1 in THF-d_8 at room temperature). No detectable NOE correlations ascribed to the *transoid*-conformation (shown in the right-hand drawing of Fig. 1(b)) could be observed under the present NMR conditions. This strongly indicates that the rotation of the acrylate is considerably restricted under the time scale of NMR (μsec order) at the range from room temperature to ~55 °C, whereas the rotation of other π-conjugated 3-vinyl- and/or 8-vinyl-substituent(s) was confirmed by the NOE correlations originating from the two different rotamers: 3^2-H (*trans* to 3^1-H) ↔ 2-CH$_3$, 3^1-H ↔ 2-CH$_3$ and 3^2-H (*trans* to 3^1-H) ↔ 5-H for the 3-vinyl group in Chl-c_1 as an example (see also Fig. S2†).

Molecular modeling calculation of Chl-c_1 and Chl-c_2

To elucidate the above rotated conformation of the 17-acrylate moiety, we performed molecular modeling calculations using a MM+/PM3 method. The central divalent magnesium of a Chl-*c* molecule was coordinated with two THF molecules as axial ligands, since THF was reported to be a hexa-coordinate solvent for most (B)Chl molecules.[19] The resulting molecules, Chl-c_1·(THF)$_2$ and Chl-c_2·(THF)$_2$, were energetically minimized. The calculated structure corresponded to the *cisoid*-conformer of the 17-acrylate as shown in the left-hand drawing of Fig. 1(b), indicating that the *cisoid*-conformer was energetically stable. To estimate total energies for the different conformers, the 17-acrylate residue was clockwise-rotated around the C17–C17^1 bond at 15° intervals (the conformation of the other peripheral substituents was fixed to that obtained for the original energy minimized). The results are depicted in Fig. S3† as a function of the rotation angle. The conformation rotated at 180°, corresponding to the *transoid*-conformation, and exhibited 7.0 kcal·mol^{-1} higher energy than that at 0° (*cisoid*) as shown in Table 1. Again, the *cisoid*-configuration was confirmed to be more thermodynamically stable

Table 1 Molecular modeling calculations of Chls-*c* by MM+/PM3

		Energy/kcal·mol^{-1}		
Substituent	Chls-*c*	*Cisoid* (0°)	*Transoid* (180°)	ΔE[a]
17-acrylate	Chl-c_1	108.5	115.5	7.0
	Chl-c_2	114.3	121.3	7.0
3-vinyl	Chl-c_1	108.5	109.7	1.2
	Chl-c_2	114.3	115.6	1.3
8-vinyl	Chl-c_2	114.3	116.0	1.7

[a] ΔE = E(*transoid*) – E(*cisoid*).

than the *transoid*, giving exclusively *cisoid* conformers at the 17-acrylate moieties from the estimated ratio of *cisoid* : *transoid* = 10^5 : 1 at room temperature. The energies for the rotamers of the 3-vinyl groups for Chls-c_1/c_2 and the 8-vinyl group for Chl-c_2 were calculated similarly. All the *cisoid* rotamers as shown in Fig. 1(a) have lower energies than the *transoid*, but the differences (ΔE) between the 0° and 180° orientations of the vinyl groups were smaller than those of the 17-acrylate: ΔE = 1.2–1.3 and 1.7 kcal·mol^{-1} for the 3- and 8-vinyl rotamers, respectively. The ratios of *cisoid* : *transoid* in vinyl groups were proposed to be 10 : 1 at most, affording the two conformers in solution at room temperature. These calculated results were consistent with those observed in NMR experiments as mentioned above.

Synthesis of Chl-*c* derivatives esterified with a phytyl group

Naturally occurring Chls-*c* exhibited lower solubilities in general organic solvents than other (B)Chls having a long hydrocarbon chain esterified at the 17^3-position, primarily due to the presence of a carboxy group in the 17-acrylate. It was thus worthwhile to synthesize the *c*-type analog having a long hydrocarbon chain at the position. This serves as an aid for investigating the detailed spectroscopic properties of *c*-type chlorophyllous pigments.

To modify Chls-*c* into more lipophilic derivatives, the carboxy group in the 17-acrylate was esterified with a C_{20} phytyl group to afford phytylated Chls-*c* as shown in Scheme 1(a). Hereafter, we denoted the phytylated Chl-*c* as "Chl-*c*-phy" using phy just after *c.* Chls-*c* were extracted from the cells of *Chaetoseros calcitrans* as mentioned above. The carboxy group of isolated Chl-c_1 and Chl-c_2 was changed to a caesium salt by CsF and esterified with phytyl bromide in DMF,[20,21] giving the desired Chls-*c*-phy in ~90% yield.

We also synthesized protochlorophyll (PChl)-*a* and its 8-vinyl analog having a propionate-type ester substituent (17-CH_2–CH_2–COO-phytyl) as shown in Scheme 1(b). PChl-*a* was quantitatively synthesized from naturally occurring Chl-*a* by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) according to reported procedures.[22] 8-Vinyl-PChl-*a* was also synthesized by DDQ oxidation of 8-vinyl-Chl-*a* extracted from the cells of Δslr1923 mutant of *Synechocystis* sp. PCC6803.[23] All the synthetic derivatives, Chl-c_1-phy, Chl-c_2-phy, PChl-*a* and 8-vinyl-PChl-*a*, were characterized by their UV-Vis, ^{1}H-NMR, infrared (IR) as well as MS spectra. From these spectra, it was established that the central magnesium, the 13-carbonyl and the 13^2-methoxy carbonyl groups remained intact during the reactions.

The effect of the acrylate moiety upon absorption and emission properties

Fig. 3 shows the electronic absorption spectra of synthetic Chls-*c*-phy in THF. Compared with the spectra of intact Chls-*c* having a carboxy group in the acrylate (see Fig. 2(b)), the synthetic corresponding esters exhibited almost identical absorption properties in terms of their spectral shapes, relative intensities and absorption maxima. The details are summarized in Table 2 and in the Experimental section. The Soret absorption maxima of natural Chls-*c* in THF were slightly more blue-shifted (~2 nm) than those of Chls-*c*-phy (the shifts of the Qx and Qy bands were less than 1 nm). This is probably due to the effect of the 17^2-substituents which were conjugated with the porphyrin π-system through the C17^1=C17^2 bond. Thus, the synthetic derivatives, showing higher solubility in general organic solvents than the corresponding intact Chls-*c*, were suitable for examination of the detailed spectroscopic properties of *c*-type chlorophylls, although the esterification decreased the fluorescence quantum yield as shown in Table 2. The fluorescence emission spectra of Chls-*c*-phy (solid) and PChls-*a* (dotted) are also indicated in the inset of Fig. 3.

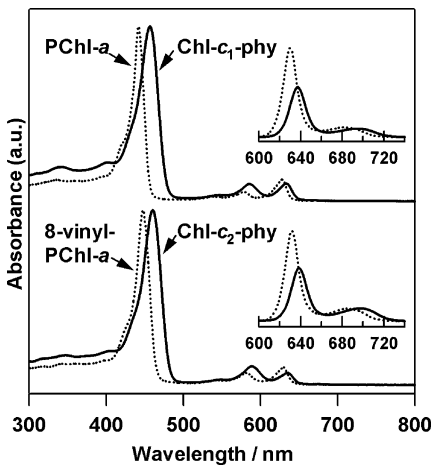


Fig. 3 Electronic absorption spectra of synthetic phytylated derivatives in THF: 8-ethyl-analogs for the upper and 8-vinyl analogs for the lower panels. The acrylate- and propionate-type derivatives are indicated by the solid and the dotted lines, respectively. All absorption spectra were normalized at the Soret maxima. Inset spectra indicate fluorescence emission spectra excited at the Soret maxima.

Table 2 Absorption and emission properties of naturally occurring Chls-*c* and the phytylated analogs, Chls-*c*-phy, and their 17^1,17^2-dihydro derivatives in THF

Compound	λ_{abs}/nm			λ_{em}[a]/nm	Quantum yield[a]/%	FWHM (Soret)/cm^{-1}
	Soret	Qx	Qy			
Chl-c_1	454.6	585.0	632.8	637.4	27	1535
Chl-c_2	458.4	588.0	634.2	639.6	23	1495
Chl-c_1-phy	456.8	585.6	633.2	637.2	8	1510
Chl-c_2-phy	460.4	588.8	634.8	638.6	7	1440
PChl-*a*	441.8	578.4	627.6	629.8	8	960
8-Vinyl-PChl-*a*	447.4	581.4	629.6	631.8	8	1060

[a] Excited at the Soret maxima.

To evaluate the effect of the acrylate moiety upon the absorption spectra, we compared the spectrum of Chl-c_1-phy (solid line in the upper trace of Fig. 3) with that of PChl-*a* (dotted line in the upper trace of Fig. 3). The structural differences between them were only in their 17-substituent (see Scheme 1). Great differences were seen in their Soret absorption. The band in Chl-c_1-phy was greatly red-shifted and broadened compared to that in PChl-*a*: 441.8 to 456.8 nm in λ_{max} and 960 to 1510 cm^{-1} in full width at half maximum (FWHM). The Qx and Qy bands were also red-shifted, but their shift and broadening were much less than that of the Soret band. The values $\Delta\lambda$s in red shift by 17^1,17^2-dehydrogenation were in the order of $\Delta\lambda$(Soret) > $\Delta\lambda$(Qx) > $\Delta\lambda$(Qy); details are summarized in Table S1.† The situation was almost identical for the 8-vinyl analogs, Chl-c_2-phy and 8-vinyl-PChl-*a*, as shown in the lower traces of Fig. 3: 447.4 to 460.4 nm in λ_{max} and 1060 to 1440 cm^{-1} in FWHM. Furthermore, we compared the absorption spectra of Chl-c_1-phy (PChl-*a*) with Chl-c_2-phy (8-vinyl-PChl-*a*) to examine the effect of the other π-conjugated vinyl group at the 8-position. The Soret absorptions of 8-vinyl analogs were also shifted to a longer wavelength than those of the corresponding 8-ethyl ones (see Table 2),[24] although the shift and broadening of the Soret absorption bands were less than those induced by the 17^1,17^2-dehydrogenation as mentioned above.

Concluding remarks

The free acrylate residue (17-CH=CH–COOH) directly conjugated with porphyrin π-system was unique in most Chls-*c* (Chl-c_1, Chl-c_2, Chl-c_3 and so on) as photosynthetically active pigments, while the same substituent was observed in other naturally occurring porphyrin pigments, such as heme d_1 and porphyrin S-411.[25] All the acrylates are well known to be *trans*-isomers but no clear evidence has been available for the rotamers. The present NMR studies unambiguously indicated that Chls-c_1 and c_2 adopted *cisoid* rotamers exclusively in solution. Considering that other unsubstituted vinyl substituents were freely rotated to be *cisoid* and *transoid* conformers, such an alternative conformer in the acrylate would be ascribable to the steric repulsion of the acrylate residue with the 13^2-$COOCH_3$. In comparison with synthetic PChls-*a* having a propionate-type ester substituent at the position, we concluded that the rigid acrylate functioned to expand the absorption region at around 400–500 nm in order to capture intense irradiation from the sun as light-harvesting pigments in photosynthesis.

Experimental

General methods

Electronic absorption spectra were measured with a Hitachi U-3500 (Hitachi, Ltd., Tokyo, Japan) spectrophotometer. Fourier-transform infrared (IR) spectra were measured with a Shimadzu FTIR-8600 spectrophotometer (Shimadzu Corporation, Kyoto, Japan); solutions were measured in a KBr cell by transmission mode. Fluorescence emission spectra were measured with a Hitachi F-4500 spectrophotometer, and emission quantum yields were measured by a photoluminescence (PL) method with an absolute PL quantum yield measurement system model C9920-02 comprising an excitation xenon light source, a monochromator, an integral sphere and a multi-channel CCD spectrometer (Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan). Optical density was about 1.0/10 mm at the Soret absorption maximum in THF for electronic absorption measurements ([Chl-*c*] = *ca.* 4.7 μM). LCMS was performed with a Shimadzu LCMS-2010EV system comprising a liquid chromatograph (SCL-10Avp system controller, LC-10ADvp pump and SPD-M10Avp photodiode array detector) and a quadrupole mass spectrometer equipped with an ESI for naturally occurring Chls-*c* and an atmospheric pressure chemical ionization (APCI) probe for the synthetic phytylated compounds as described previously.[26] High-resolution FAB mass spectra were measured with a JEOL GC-Mate II (JEOL, Ltd., Akishima, Tokyo). ^{1}H- and ^{13}C-NMR spectra were measured by a JEOL ECA-600 NMR spectrometer in THF-d_8 at room temperature. A set of assignments of both ^{1}H and ^{13}C signals was obtained using ^{1}H-^{1}H COSY and ^{1}H-^{1}H NOESY (τ_m = 600 msec) for ^{1}H signals and distortionless enhancement polarization transfer (DEPT), ^{1}H-^{13}C COSY and ^{1}H-^{13}C HMBC ($^nJ_{CH}$ = 10 Hz) for ^{13}C signals. All solvents were commercially available and used without further purification.

Preparation of Chls-*c*

A commercially available diatom, *Chaetoseros calcitrans*, was purchased from Nisshin Marinetech Co., Ltd. (Yokohama, Japan) as an active culture. Chls-*c*, a mixture of Chl-c_1 and Chl-c_2, was extracted from the above diatom as follows. A mixture of acetone, methanol and petroleum ether was added to the harvested cells, stirred for 15 min in the dark, and filtered to remove hydrophobic components including Chl-*a* and carotenoids. The residue was re-extracted with a mixture of pyridine and chloroform to give crude Chls-*c*. Here, pyridine was a good solvent for the extraction, but the solvent induced rapid epimerization at the 13^2-position.[15] The extracted Chls-*c* were purified by silica-gel chromatography (Wakogel C-300, Wako Pure Chemical Industries, Ltd., Osaka, Japan) to remove undesired components, and recrystallized from hexane to give pure Chls-*c* as a c_1 and c_2 mixture. The ratio of Chl-c_1 and Chl-c_2 was almost 1 : 1 as estimated by HPLC (the value was slightly dependent on the batch of diatom purchased).

Separation of Chl-c_1 and Chl-c_2

To separate Chl-c_1 and Chl-c_2, reverse-phase HPLC using a polymeric ODS column was applied according to reported methods[9,12,18] with modification as follows: column, Inertsil ODS-P (4.6 × 150 mm for analysis and 10 × 250 mm for preparation; GL Sciences Inc., Tokyo); eluent, methanol : acetonitrile : 50 mM ammonium acetate (pH 5.25) = 400 : 75 : 25 (v/v/v). To identify Chl-c_1 and Chl-c_2, LCMS equipped with an ESI probe in combination with the above HPLC was used. Both samples were enantiomeric mixtures due to the presence of sole and readily isomerizing chiral carbon at the 13^2-position.

Chl-c_1. λ_{max}(THF)/nm 632 (relative intensity, 0.11), 584 (0.10) and 454 (1.00); δ_H(600 MHz; THF-d_8; Me_4Si) 10.06 (1H, s, 10-H), 10.00 (1H, s, 20-H), 9.97 (1H, s, 5-H), 9.09 (1H, d, J = 15.8 Hz, 17^1-H), 8.35 (1H, dd, J = 11.5 Hz, 17.8 Hz, 3^1-H), 6.96 (1H, s, 13^2-H), 6.71 (1H, d, J = 15.8 Hz, 17^2-H), 6.38 (1H, d, J = 17.9 Hz, 3^2-H *trans* to 3^1-H), 6.07 (1H, d, J = 11.3 Hz, 3^2-H *cis* to 3^1-H), 4.02 (2H, q, J = 7.8 Hz, 8-CH_2), 3.83 (3H, s, 12-CH_3), 3.77

(3H, s, 13^2-$COOCH_3$), 3.71 (3H, s, 18-CH_3), 3.68 (3H, s, 2-CH_3), 3.52 (3H, s, 7-CH_3) and 1.83 (3H, t, J = 7.8 Hz, 8^1-CH_3) [17^2-COO*H* was not identified due to the fast exchange with a trace contaminant H_2O in the solvent]; δ_C(150 MHz; THF-d_8; Me_4Si) 188.8 ($C13^1$), 170.6 ($C13^3$), 167.1 ($C17^3$), 158.3 (C14 or C16), 152.3 (C6), 151.9 (C11), 149.4 (C19), 149.2 (C9), 149.1, 149.0, 143.6 (C1, C4 and C14 or C16), 144.6 (C8), 141.5 (C18), 138.8 ($C17^1$), 138.4 (C2), 138.1 (C3), 136.3 (C7), 135.5 (C13), 135.0 (C12), 134.7 (C17), 130.9 ($C3^1$), 123.8 ($C17^2$), 118.7 ($C3^2$), 112.4 (C15), 101.5 (C10), 98.2 (C20), 97.9 (C5), 67.9 ($C13^2$), 51.6 ($C13^4$), 19.5 ($C8^1$), 17.2 ($C8^2$), 12.9 ($C18^1$), 12.1 ($C2^1$), 11.9 ($C12^1$) and 10.6 ($C7^1$); *m/z* (ESI) 611.3 (MH^+).

Chl-c_2. λ_{max}(THF)/nm 634 (relative intensity, 0.08), 587 (0.11) and 458 (1.00); δ_H(600 MHz; THF-d_8; Me_4Si) 10.21 (1H, s, 10-H), 10.07 (1H, s, 5-H), 10.03 (1H, s, 20-H), 9.10 (1H, d, J = 16.0 Hz, 17^1-H), 8.39 (2H, dd, J = 11.5, 17.9 Hz, 3^1-, 8^1-H), 6.98 (1H, s, 13^2-H), 6.74 (1H, d, J = 16.0 Hz, 17^2-H), 6.41 (1H, d, J = 18.2 Hz, 3^2-H *trans* to 3^1-H), 6.38 (1H, d, J = 17.9 Hz, 8^2-H *trans* to 8^1-H), 6.11 (1H, d, J = 10.9 Hz, 3^2-H *cis* to 3^1-H), 6.09 (1H, d, J = 11.2 Hz, 8^2-H *cis* to 8^1-H), 3.84 (3H, s, 12-CH_3), 3.79 (3H, s, 13^2-$COOCH_3$), 3.73 (3H, s, 18-CH_3), 3.71 (3H, s, 2-CH_3) and 3.69 (3H, s, 7-CH_3) [17^2-COO*H* was not identified due to the fast exchange with a trace contaminant H_2O in the solvent]; δ_C(150 MHz; THF-d_8; Me_4Si) 188.8 ($C13^1$), 170.5 ($C13^3$), 167.1 ($C17^3$), 158.5 (C14 or C16), 152.0 (C11), 151.3 (C6), 149.5 (C19), 149.3 (C1), 148.9, 148.4, 144.0 (C4, C9 and C14 or C16), 141.8 (C18), 138.8 (C8), 138.7 ($C17^1$), 138.4 (C2), 138.3 (C3), 137.5 (C7), 135.64, 135.60 (C12 and C13), 134.9 (C17), 130.94 ($C8^1$), 130.88 ($C3^1$), 123.9 ($C17^2$), 118.83, 118.77 ($C3^2$, $C8^2$), 112.4 (C15), 102.5 (C10), 98.4 (C5), 98.3 (C20), 67.8 ($C13^2$), 51.6 ($C13^4$), 12.9 ($C18^1$), 12.12 ($C7^1$), 12.08 ($C2^1$) and 11.98 ($C12^1$); *m/z* (ESI) 609.2 (MH^+).

Synthesis of Chls-*c*-phy (acrylate-type derivatives)

1-Bromo-3,7,11,15-tetramethylhexadec-2-ene (phytyl bromide) was synthesized according to reported methods.[20] Phosphorus tribromide (72 μL) was added to a solution of phytol (*Z* + *E*) (460 mg) in hexane (5 mL) at 0 °C. The mixture was stirred under N_2 gas for 10 min, and then methanol (0.2 mL) was added and further stirred for 5 min. The reaction mixture was washed with aqueous 4% (wt/v) $NaHCO_3$ and distilled water, dried over anhydrous $MgSO_4$, and concentrated. The crude product was used in the following step.

Chl-c_1 was esterified with phytyl bromide similarly to reported procedures.[21] Crude phytyl bromide (30 mg) and CsF (8.1 mg) were added to a solution of Chl-c_1 (16 mg) in DMF (5 mL). The mixture was stirred under N_2 gas overnight. The reaction mixture was washed with aqueous saturated NaCl and distilled water, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography (Wakogel C-300, 3–5% acetone in CH_2Cl_2) to give Chl-c_1-phy in 91% yield. Chl-c_2 was also esterified by the same method (86%).

Chl-c_1-phy. ν_{max}(THF)/cm^{-1} 1738 (ester-C=O) and 1707 (keto-C=O); λ_{max}(THF)/nm 633 (relative intensity, 0.10), 586 (0.10) and 457 (1.00); δ_H(600 MHz; THF-d_8; Me_4Si) 10.08 (1H, s, 10-H), 10.03 (1H, s, 20-H), 9.99 (1H, s, 5-H), 9.16 (1H, d, J = 16.1 Hz, 17^1-H), 8.37 (1H, dd, J = 11.3, 17.8 Hz, 3^1-H), 7.01 (1H, s, 13^2-H), 6.78 (1H, d, J = 15.8 Hz, 17^2-H), 6.40 (1H, d, J = 17.9 Hz, 3^2-H *trans* to 3^1-H), 6.09 (1H, d, J = 11.3 Hz, 3^2-H *cis* to 3^1-H), 5.64 (1H, t, J = 6.7 Hz, phytyl 2-H), 4.94 (2H, m, phytyl 1-H_2), 4.03 (2H, q, J = 7.6 Hz, 8-CH_2), 3.85 (3H, s, 12-CH_3), 3.77 (3H, s, 13^2-$COOCH_3$), 3.73 (3H, s, 18-CH_3), 3.70 (3H, s, 2-CH_3), 3.54 (3H, s, 7-CH_3), 2.14 (2H, m, phytyl 4-H_2), 1.89 (3H, s, phytyl 3-CH_3), 1.84 (3H, t, J = 7.7 Hz, 8^1-CH_3), 1.61–1.06 (18H, m, phytyl 5-, 6-, 8-, 9-, 10-, 12-, 13-, 14-H_2, 7-, 11-H), 1.52 (1H, m, phytyl 15-H), 0.92, 0.87 (each 3H, d, J = 6.5 Hz, phytyl 7-, 11-CH_3) and 0.86 (6H, d, J = 6.5 Hz, phytyl 15-CH_3, 16-H_3) [the numbering of phytyl protons corresponded to that in Scheme 1(a)]; *m/z* (FAB) 888.5063 (M^+. $C_{55}H_{68}N_4O_5Mg$ requires 888.5040).

Chl-c_2-phy. ν_{max}(THF)/cm^{-1} 1738 (ester-C=O) and 1707 (keto-C=O); λ_{max}(THF)/nm 635 (relative intensity, 0.07), 589 (0.11) and 460 (1.00); δ_H(600 MHz; THF-d_8; Me_4Si) 10.25 (1H, s, 10-H), 10.08 (1H, s, 5-H), 10.08 (1H, s, 20-H), 9.18 (1H, d, J = 15.8 Hz, 17^1-H), 8.39 (2H, dd, J = 11.6, 17.8 Hz, 3^1-, 8^1-CH), 7.04 (1H, s, 13^2-H), 6.80 (1H, d, J = 15.8 Hz, 17^2-H), 6.40 (1H, d, J = 17.4 Hz, 3^2-H *trans* to 3^1-H), 6.38 (1H, d, J = 17.5 Hz, 8^2-H *trans* to 8^1-H), 6.10, 6.09 (each 1H, d, J = 11.4 Hz, 3^2-, 8^2-H *cis* to 3^1-, 8^1-H), 5.63 (1H, t, J = 7.0 Hz, phytyl 2-H), 4.94 (2H, m, phytyl 1-H_2), 3.85 (3H, s, 12-CH_3), 3.78 (3H, s, 13^2-$COOCH_3$), 3.74 (3H, s, 18-CH_3), 3.71 (3H, s, 2-CH_3), 3.65 (3H, s, 7-CH_3), 2.14 (2H, m, phytyl 4-H_2), 1.89 (3H, s, phytyl 3-CH_3), 1.62–1.07 (18H, m, phytyl 5-, 6-, 8-, 9-, 10-, 12-, 13-, 14-H_2, 7-, 11-H), 1.52 (1H, m, phytyl 15-H), 0.92, 0.87 (each 3H, d, J = 6.5 Hz, 7-, 11-H_3) and 0.86 (6H, d, J = 6.5 Hz, phytyl 15-CH_3, 16-H_3) [the numbering of phytyl protons corresponded to that in Scheme 1(a)]; *m/z* (FAB) 886.4853 (M^+. $C_{55}H_{66}N_4O_5Mg$ requires 886.4884).

Synthesis of PChls-*a* (propionate-type derivatives)

As reported earlier, Chl-*a* was extracted from *Spirulina geitleri*.[27] The C17–C18 single bond in Chl-*a* was quantitatively oxidized with DDQ in acetone to afford protochlorophyll (PChl)-*a*, according to reported procedures.[22] DDQ in acetone (*ca.* 9 mM) was added to an acetone solution (10 mL) of Chl-*a*(/*a*′) (~8 : 2, 20 mg) and the mixture was stirred at room temperature. After disappearance of the 661 nm peak characteristic of the chlorin moiety, the reaction mixture was extracted with CH_2Cl_2 and aqueous 1% (wt/v) $KHSO_4$. The organic phase was washed with aqueous 4% (wt/v) $NaHCO_3$ and distilled water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography on silica gel (Wakogel C-300, 2–5% (v/v) acetone in CH_2Cl_2) and recrystallized from hexane, yielding an enantiomeric mixture of PChl-*a* as a green solid (16 mg, 80%): ν_{max}(THF)/cm^{-1} 1740 (ester-C=O) and 1703 (keto-C=O); λ_{max}(THF)/nm 628 (relative intensity, 0.12), 577 (0.05) and 442 (1.00); δ_H(600 MHz; THF-d_8; Me_4Si) 10.10 (1H, s, 10-H), 9.98 (1H, s, 5-H), 9.92 (1H, s, 20-H), 8.37 (1H, dd, J = 11.5, 17.7 Hz, 3^1-H), 7.02 (1H, s, 13^2-H), 6.39 (1H, d, J = 17.8 Hz, 3^2-H *trans* to 3^1-H), 6.08 (1H, d, J = 11.3 Hz, 3^2-H *cis* to 3^1-H), 5.33 (1H, t, J = 6.5 Hz, phytyl 2-H), 4.60 (2H, d, J = 6.8 Hz, phytyl 1-H_2), 4.19, 4.11 (each 1H, m, 17-CH_2), 4.04 (2H, q, J = 7.6 Hz, 8-CH_2), 3.87 (3H, s, 12-CH_3), 3.73 (3H, s, 13^2-$COOCH_3$), 3.69 (3H, s, 2-CH_3), 3.56 (3H, s, 18-CH_3), 3.55 (3H, s, 7-CH_3), 3.10, 2.92 (each 1H, m, 17^1-CH_2), 1.99 (2H, m, phytyl 4-H_2), 1.85 (3H, t, J = 7.6 Hz, 8^1-CH_3), 1.68 (3H, s, phytyl 3-CH_3), 1.52 (1H, m, phytyl 15-H), 1.47–1.02 (18H, m, phytyl 5-, 6-, 8-, 9-, 10-, 12-, 13-, 14-H_2, 7-, 11-H) and 0.86 (12H, d, J = 6.5 Hz, phytyl 7-, 11-,

15-CH_3,16-H_3) [the numbering of phytyl protons corresponded to that in Scheme 1(a)]; *m/z* (APCI) 891.5 (MH^+).

Synthesis of 8-vinyl-PChl-*a*

Δslr1923 mutant of *Synechocystis* sp. PCC6803 exclusively containing 8-vinyl-Chl-*a* was provided by Prof. A. Tanaka (Hokkaido University, Japan) and was cultured aerobically as described previously.[23] 8-Vinyl-Chl-*a* was extracted from the harvested cells of Δslr1923 mutant of *Synechocystis* sp. PCC6803 similarly as described previously.[26] The C17–C18 single bond in 8-vinyl-Chl-*a* was oxidized with DDQ in acetone to afford an enantiomeric mixture of 8-vinyl-PChl-*a*, similar to the synthesis of PChl-*a* mentioned above: ν_{max}(THF)/cm^{-1} 1740 (ester-C=O) and 1705 (keto-C=O); λ_{max}(THF)/nm 630 (relative intensity, 0.10), 581 (0.07) and 447 (1.00); δ_H(600 MHz; THF-d_8; Me_4Si) 10.25 (1H, s, 10-H), 10.06 (1H, s, 5-H), 9.93 (1H, s, 20-H), 8.40, 8.39 (each 1H, dd, $J = 11.3$, 17.9 Hz, 3^1-, 8^1-CH), 7.03 (1H, s, 13^2-H), 6.40 (1H, d, $J = 17.9$ Hz, 3^2-H *trans* to 3^1-H), 6.37 (1H, d, $J = 17.9$ Hz, 8^2-H *trans* to 8^1-H), 6.09, 6.08 (each 1H, d, $J = 11.3$ Hz, 3^2-, 8^2-H *cis* to 3^1-, 8^1-H), 5.32 (1H, t, $J = 6.8$ Hz, phytyl 2-H), 4.59 (2H, d, $J = 6.8$ Hz, phytyl 1-H_2), 4.20, 4.12 (each 1H, m, 17-CH_2), 3.86 (3H, s, 12-CH_3), 3.73 (3H, s, 13^2-$COOCH_3$), 3.69 (3H, s, 2-CH_3), 3.68 (3H, s, 7-CH_3), 3.52 (3H, s, 18-CH_3), 3.10, 2.92 (each 1H, m, 17^1-CH_2), 1.98 (2H, m, phytyl 4-H_2), 1.67 (3H, s, phytyl 3-CH_3), 1.51 (1H, m, phytyl 15-H), 1.56–1.03 (18H, m, phytyl 5-, 6-, 8-, 9-, 10-, 12-, 13-, 14-H_2, 7-, 11-H) and 0.86, 0.85 (12H, d, $J = 6.5$ Hz, phytyl 7-, 11-, 15-CH_3, 16-H_3) [the numbering of phytyl protons corresponded to that in Scheme 1(a)]; *m/z* (FAB) 888.5069 (M^+. $C_{55}H_{68}N_4O_5Mg$ requires 888.5040).

Molecular modeling calculations

Molecular modeling calculations of Chls-*c* were performed using the MM+/PM3 method in a HYPERCHEM version 6.01 software package (Hypercube Inc., Florida, USA).[28,29] The central divalent magnesium of a Chl-*c* molecule was coordinated with two THF molecules as axial ligands. Chl-c_1·$(THF)_2$ and Chl-c_2·$(THF)_2$ were energetically minimized by the above calculation until the energy gradient reached less than 0.01 kcal·mol^{-1}. The resulting molecular model, which corresponded to the *cisoid*-conformation at the 17-position, was used for estimating the energy of different configurations of the 17-acrylate around the C17–$C17^1$ bond at a clockwise rotation of a 15° interval. The energy of the rotation of the 3-vinyl group for Chl-c_1 and the 3,8-divinyl groups for Chl-c_2 was also calculated in a way similar to the acrylate mentioned above.

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