Bioorganic & Medicinal Chemistry 19 (2011) 52–57

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Reduction of vinyl groups in naturally occurring chlorophylls-*a*

Hitoshi Tamiaki *, Daisuke Takekoshi, Tadashi Mizoguchi

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

ARTICLE INFO

Article history:
Received 5 November 2010
Revised 23 November 2010
Accepted 23 November 2010
Available online 30 November 2010

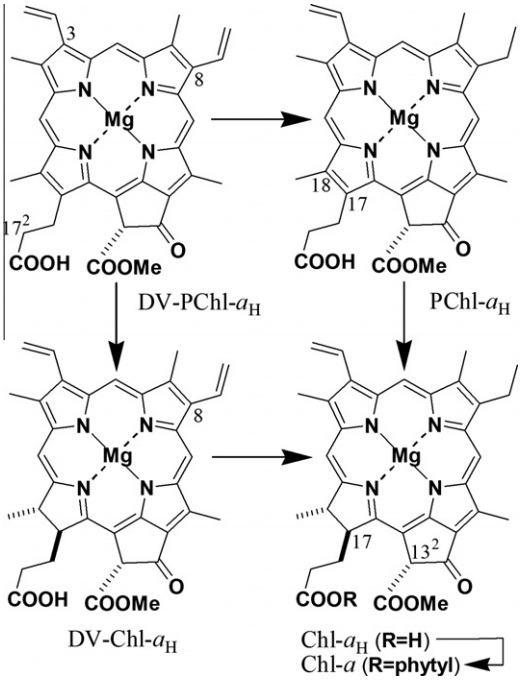
Keywords:
Biosynthesis
Hydrogenation
Regioisomer
Site-selectivity
Substitution effect

ABSTRACT

3,8-Divinyl-chlorophyll(Chl)-*a* possessing a phytyl ester was hydrogenated in acetone by rhodium catalyst on alumina to afford 3-vinyl-8-ethyl-, 3-ethyl-8-vinyl- and 3,8-diethyl-Chls. The ratio of produced 3-ethyl-8-vinyl- over 3-vinyl-8-ethyl-Chls was determined to be 1.2, indicating that the reactivity of the 3-vinyl group was slightly higher than that of the 8-vinyl group. Catalytic hydrogenation of divinyl-protochlorophyll-*a* possessing a porphyrin π-skeleton (C17=C18) instead of the above chlorin moiety (C17H–C18H) gave an equal amount of mono-reduced regioisomers. The slight (or no) selectivity is different from that in the enzymatic reduction of divinyl-(proto)chlorophyllides-*a* lacking a phytyl ester in the biosynthetic pathway of Chl-*a* where the sole 8-vinyl group is transformed to the ethyl group.

1. Introduction

Chlorophyll(Chl)-*a* is one of the most abundant pigments in natural systems,[1] functioning as a light-absorbing, energy-transferring and electron-transporting component in the photosynthetic antenna and reaction center apparatus.[2] The biosynthesis of Chl-*a* has been widely investigated and most of the engaging enzymes were identified.[3] In the last stages of the Chl-*a* biosynthetic pathway (see Scheme 1), divinyl-protochlorophyllide-*a* (DV-PChl-a_H) is selectively hydrogenated at the 8-vinyl group (not the 3-vinyl group) by a divinyl reductase with NADPH to afford protochlorophyllide-*a* (PChl-a_H),[4,5] which is further hydrogenated at the C17=C18 moiety by a light-(in)dependent oxidoreductase, (D)POR, to chlorophyllide-*a* (Chl-a_H).[6] Chl-a_H is esterified by geranylgeranyl diphosphate (by chlorophyll synthase) and reduced regioselectively at three of the four C=C bonds in the esterifying moiety (by geranylgeranyl reductase) to Chl-*a* possessing a phytyl ester at the 17-propionate residue: direct esterification of Chl-a_H with phytyl diphosphate would give Chl-*a* as well.[2,7] Recently, an alternative route was proposed for the in vivo 8-vinyl reduction. The C17=C18 of DV-PChl-a_H is first reduced to divinyl-chlorophyllide-*a* (DV-Chl-a_H) and successively the 8-CH=CH_2 of DV-Chl-a_H is reduced to Chl-a_H.[5,8] The hydrogenation of the vinyl group at the peripheral position of both the cyclic tetrapyrroles, porphyrin

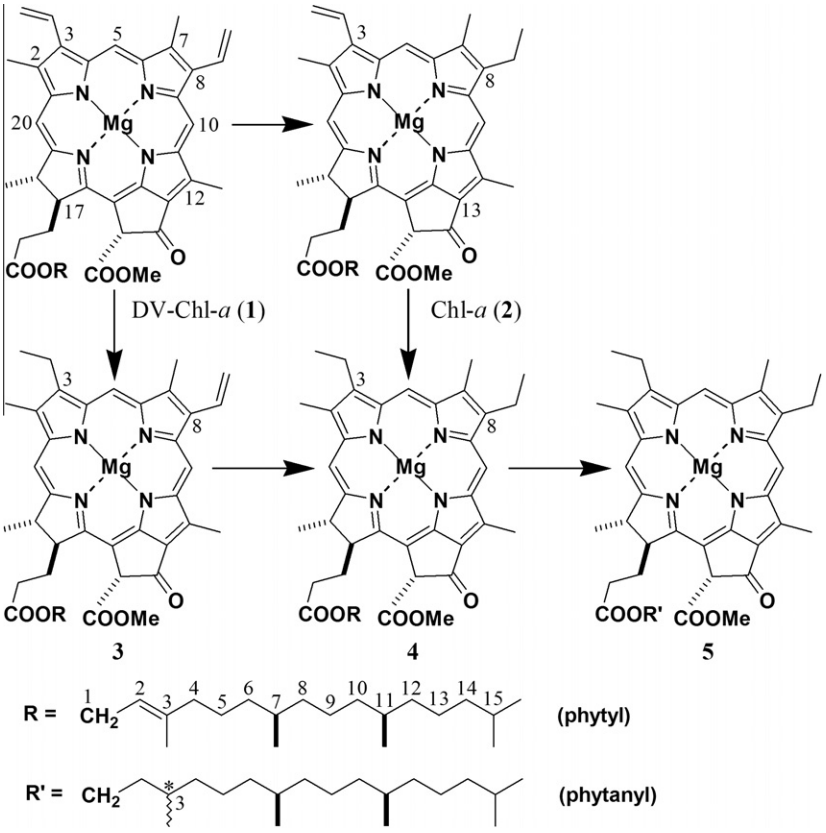


Scheme 1. Biosynthetic pathway from divinyl-chlorophyllide-*a* (DV-Chl-a_H) to chlorophyll(ide)-*a* [Chl-$a_{(H)}$]. The subscript H indicates the hydrogen atom of the 17^2-carboxy group.

Abbreviations: APCI, atmospheric pressure chemical ionization; BChl, bacteriochlorophyll; Chl, chlorophyll; DV, divinyl; ESI, electron-spray ionization; FCC, flash column chromatography; PChl, protochlorophyll; (P)Chl-a_H, (proto)chlorophyllide-*a*; TOF, time-of-flight.

* Corresponding author. Tel.: +81 77 561 2765; fax: +81 77 561 2659.
E-mail address: tamiaki@se.ritsumei.ac.jp (H. Tamiaki).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.11.056



Scheme 2. Stepwise hydrogenation of divinyl-chlorophyll-*a* (DV-Chl-*a*, **1**) to mesochlorophylls-*a* **4**/**5** through monovinyl-chlorins **2** and **3**.

(DV-PChl-a_H) and chlorin (DV-Chl-a_H) proceeds site-selectively and the transformation of 8-vinyl to 8-ethyl groups is observed exclusively in the biosynthetic pathways.

All the naturally occurring (bacterio)chlorophylls are biosynthesized through the common precursor, DV-PChl-a_H.[3] As in Chl-*a* mentioned above, the 8-vinyl group is reduced in the biosynthetic pathways of the other Chls, except for DV-Chl-*a*, DV-Chl-*b*, Chl-c_2 and Chl-c_3.[2,3] Most bacteriochlorophylls (BChls) found in photosynthetic bacteria are also produced through the selective reduction: BChls-*a*, *c*, *d* and *e*.[2,3] In the later biosynthetic routes, the remaining reactive 3-vinyl group is often modified to the other substituents: 3-CHO in Chl-*d*, 3-COMe in BChl-*a*, and 3-CH(OH)Me in BChls-*c*/*d*/*e*.[3]

The above enzymatic reduction gives exclusively 3-vinyl-8-ethyl-compounds, but the chemical selectivity in the hydrogenation of DV-(P)Chl-a_H as the substrate has not yet been investigated. Here, catalytic hydrogenation of phytylated DV-(P)Chl-*a* (**1**/**6**) in acetone was examined and the reactivity of the 3- and 8-vinyl groups was revealed. It is noted to prepare 8-vinyl-mesochlorophyll-*a* (**3**) possessing the 3-ethyl and 8-vinyl groups for the first time (see Scheme 2), which is the novel isomer of Chl-*a* (**2**) possessing the 3-vinyl and 8-ethyl groups other than the 13^2-epimer of Chl-*a*, (13^2S)-Chl-*a*′.[2]

2. Results and discussion

2.1. Catalytic hydrogenation of divinyl-chlorophyll-*a*

The 3-vinyl group of Chl-*a* (**2**) was reported to be reduced in acetone by the catalytic action of palladium on charcoal under hydrogen atmosphere to afford selectively the corresponding 3-ethyl-chlorin, mesochlorophyll-*a* (**4**), without reduction of the 13-keto carbonyl group.[9] Under the same conditions, 3,8-divinyl-chlorophyll-*a* (DV-Chl-*a*, **1**) was hydrogenated to give 3,8-diethyl-chlorin **4** after quick double reduction of the 3- and 8-vinyl groups because Pd/C was one of the effective catalysts for hydrogenation. Another insoluble catalyst, rhodium on alumina was found to be moderate for the present hydrogenation, while soluble $RhCl(PPh_3)_3$ was less reactive. Here, Rh/Al_2O_3 was used as the hydrogenation catalyst to examine the stepwise reduction of the double bonds in naturally occurring DV-Chl-*a* (**1**).

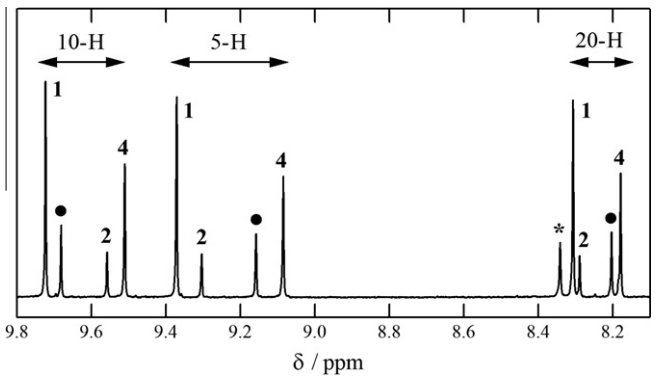


Figure 1. Partial ^{1}H NMR spectrum of a hydrogenated mixture of divinyl-chlorophyll-*a* (DV-Chl-*a*, **1**) in 1% pyridine-d_5/$CDCl_3$, containing **1**, **2**, **3** (•) and **4**. Peak ∗ at 8.34 ppm was assigned to *o*-H of C_5HD_4N.

DV-Chl-*a* (**1**) was dissolved in acetone, to which Rh/Al_2O_3 was added. After the mixture was stirred under H_2 at room temperature for 1 h, the catalyst was removed by filtration and the solvent of the filtrate was evaporated. The residue was analyzed by ^{1}H NMR spectroscopy and the spectrum at the low-field region between

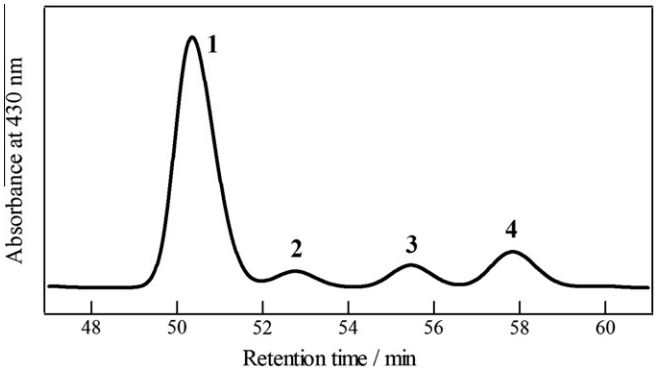


Figure 2. HPLC of a hydrogenated mixture of divinyl-chlorophyll-*a* (DV-Chl-*a*, **1**) containing **1**–**4**: column, Cosmosil $5C_{18}$-AR-II 4.6 $\phi \times 150$ mm; eluent, water/methanol/acetonitrile = 1/19/20; flow rate, 1.0 ml/min.

8.1 and 9.8 ppm is shown in Figure 1. The peaks are ascribed to *meso*-protons, 5-, 10- and 20-protons. In comparison to the *meso*-peaks of the three authentic samples, 3,8-divinyl- (**1**), 3-vinyl-8-ethyl- (**2**) and 3,8-diethyl-chlorins (**4**), one set of the three peaks shown in closed circles of Figure 1 remained and another chlorophyll-*a* analog was produced during the hydrogenation. The reaction mixture was checked by RP-HPLC and four chlorophylls-*a* were successfully separated using an octadecylated silica gel column and water/methanol/acetonitrile = 1/19/20 as an eluent (see Figure 2). The first, second and fourth eluted bands were assigned to **1**, **2** and **4**, respectively. The third band was separated and the sample was analyzed by various spectroscopies. The main peaks of the mass spectrum obtained by electron-spray ionization (ESI) were at m/z = 893.7 and 614.2, which were the same as the values for Chl-*a* (**2**). The former peak came from MH^+ and the latter was from $[MH-phytyl]^+$. Therefore, the third fraction should be a phytylated chlorophyll-*a* isomer produced by reduction of one double bond conjugated with the chlorin π-system in DV-Chl-*a* (**1**).

The ^1H NMR spectrum in $CDCl_3$ containing 1% pyridine-d_5 showed that the third separated sample had one vinyl group and one ethyl group at the peripheral positions. The substituent pattern was determined by the following comparison of chemical shifts (δs). The reduction of 3- and 8-vinyl groups as in **2** to **4** and **1** to **2**, respectively, changes δs of the *meso* and peripheral methyl protons: 5-, 10-, 20-H and 2-, 7-, 12-CH_3. Their δs of 8-vinyl-mesochlorophyll-*a* (**3**) were proposed from changes of δs by hydrogenation of the 3-vinyl group in **1** as well as by dehydrogenation of the 8-ethyl group in **4**. The calculated δs based on the additivity were almost the same as the values observed for the third eluted sample (see Table 1). The third fraction was thus assigned to 3-ethyl-8-vinyl-chlorin **3**.

The visible spectrum of the third eluted sample in dichloromethane was compared with those of the other three chlorophylls-*a* (see Figure 3). The reduction of 3- and 8-vinyl groups

Table 1
Chemical shifts (δs) of partial protons in chlorophylls-*a*[†]

	δ/ppm				
	1 [3V,8V]	**2** [3V,8E]	**3**[‡] [3E,8V]	**4** [3E,8E]	**5** [3E,8E; phytanyl]
2-Me	3.30	3.30	3.16 (3.15)	3.15	3.15
5-H	9.37	9.29	9.15 (9.16)	9.08	9.08
7-Me	3.38	3.24	3.38 (3.38)	3.24	3.24
10-H	9.72	9.55	9.68 (9.68)	9.51	9.51
12-Me	3.64	3.65	3.63 (3.63)	3.64	3.63
20-H	8.30	8.28	8.20 (8.20)	8.18	8.18

[†] The δs were measured in 1% pyridine-d_5/$CDCl_3$.
[‡] The value in parenthesis indicates expected data from the values of **1**, **2** and **4**: calculated $\delta(\mathbf{3}) = \delta(\mathbf{1}) + [\delta(\mathbf{4}) - \delta(\mathbf{2})]$ or $\delta(\mathbf{4}) + [\delta(\mathbf{1}) - \delta(\mathbf{2})]$.

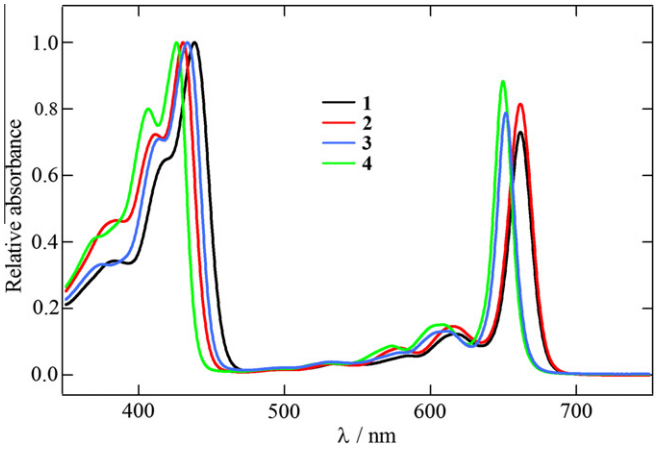


Figure 3. Visible absorption spectra of divinyl-chlorophyll-*a* (DV-Chl-*a*, **1**), chlorophyll-*a* (Chl-*a*, **2**), 8-vinyl-mesochlorophyll-*a* (**3**) and mesochlorophyll-*a* (**4**) in dichloromethane. All the spectra were normalized at the Soret maxima.

Table 2
Absorption maxima (λ_{max}) of chlorophylls-*a* in CH_2Cl_2

	λ_{max}/nm				
	1 [3V,8V]	**2** [3V,8E]	**3**[†] [3E,8V]	**4** [3E,8E]	**5** [3E,8E; phytanyl]
Qy	662	660	652 (652)	650	650
Soret	438	430	433 (433)	425	425

[†] The value in parenthesis indicates expected data from the values of **1**, **2** and **4**: calculated $\lambda_{max}(\mathbf{3}) = \lambda_{max}(\mathbf{1}) + [\lambda_{max}(\mathbf{4}) - \lambda_{max}(\mathbf{2})]$ or $\lambda_{max}(\mathbf{4}) + [\lambda_{max}(\mathbf{1}) - \lambda_{max}(\mathbf{2})]$.

shifted the Qy and Soret absorption maxima (λ_{max}) to shorter wavelengths. The blue shifts of Qy and Soret peaks were 10 and 5 nm, respectively, for the hydrogenation of the 3-vinyl group as in **2** to **4**, while their red-shifts were 2 and 8 nm for the dehydrogenation of the 8-ethyl group as in **1** to **2** (see Table 2). The estimated λ_{max} for **3** were 652 (=662 − 10 or 650 + 2) and 433 nm (=438 − 5 or 425 + 8), which were identical to the experimental data. The consistency confirmed that the third fraction was 3-ethyl-8-vinyl-chlorin **3**.

The above results indicate that **1** was hydrogenated under the present conditions to give **2**, **3** and **4** without epimerization at the 13^2-position and reduction the C7=C8. Prolonged hydrogenation afforded phytanylated mesochlorophyll-*a* (**5**, see Scheme 2). The three-substituted double bond of the phytyl group was less

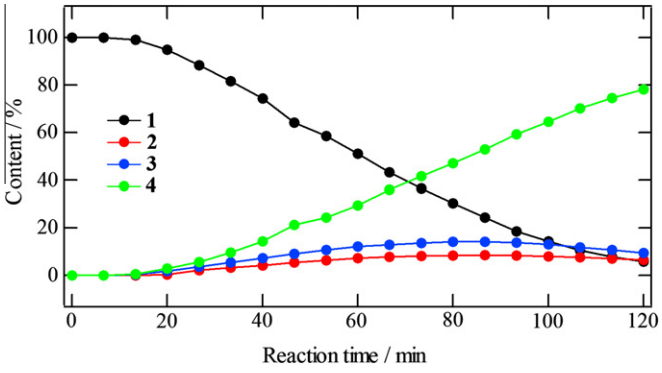


Figure 4. Time-dependent contents of divinyl-chlorophyll-*a* (DV-Chl-*a*, **1**), chlorophyll-*a* (Chl-*a*, **2**), 8-vinyl-mesochlorophyll-*a* (**3**) and mesochlorophyll-*a* (**4**) during the stepwise hydrogenation of **1** by the catalytic action of Rh/Al_2O_3 in acetone, determined from HPLC analysis using their relative extinction coefficients at 660 nm (see Section 4.2).

reactive due to the highly steric hindrance, so the two vinyl groups at the 3- and 8-positions were first reduced and then the phytyl group of the resulting **4** was hydrogenated to the phytanylated compound **5**. Since the present hydrogenation proceeded non-stereochemically, the stereochemistry at the 3-position of phytanyl group would be racemic.

2.2. Hydrogenation pathways of divinyl-chlorophyll-*a*

During the hydrogenation of **1**, three chlorophylls, **2**, **3** and **4**, were observed as the products, indicating that the 3- and 8-vinyl groups were stepwise hydrogenated. The hydrogenation was monitored by HPLC and the ratios of the four chlorophylls-*a* were determined (see Figure 4). Divinyl-chlorin **1** gradually decreased and concomitantly diethyl-chlorin **4** increased to 80% after hydrogenation for 2 h. Both regioisomeric monovinyl-monoethyl-chlorins **2** and **3** were observed and their components reached maxima at ca. 1.5 h. The content of 8-vinyl-chlorin **3** was always larger than that of 3-vinyl-chlorin **2**. The ^{1}H NMR spectrum shown in Figure 1 also supports the difference: *meso*-peaks of **3** were slightly larger than those of **2**. The difference in the contents of the two monovinyl-chlorophylls can be explained by the slight enhancement of hydrogenation reactivity at the 3-vinyl group over that at the 8-vinyl group. The 3-vinyl group of **1** was reduced slightly more rapidly than the 8-vinyl group and/or the 3-vinyl group of **2** more than the 8-vinyl group of **3**.

A mixture of isomeric monovinyl-chlorin **2** and **3** was hydrogenated under the same conditions described above and the ratio was analyzed by HPLC. During the hydrogenation, **2** and **3** decreased and **4** increased (Figure 5A). The ratio of **2** over **3** was faintly changed for the whole reaction period (Figure 5B), indicating that the hydrogenation reactivity of the 3-vinyl group in **2** was almost the same as that of the 8-vinyl group in **3**. Therefore, the above difference in the content of monovinyl-chlorophylls-*a* at the hydrogenation of DV-Chl-*a* (**1**) shows that the 3-vinyl group of **1** was slightly more reactive than the 8-vinyl group. The present difference in the regioselective reduction of C=C at the 3- over 8-positions is at most 1.2 times for the product ratio, while the 3-C=O of chlorophyll derivatives was reported to be reduced at least 5 times more rapidly than the 8-C=O.[10] It is noteworthy that similar reactivities of the 3- and 8-vinyl groups in catalytic hydrogenation are distinguishable from the large reduction reactivity of the 3-carbonyl group over that of the 8-carbonyl group.

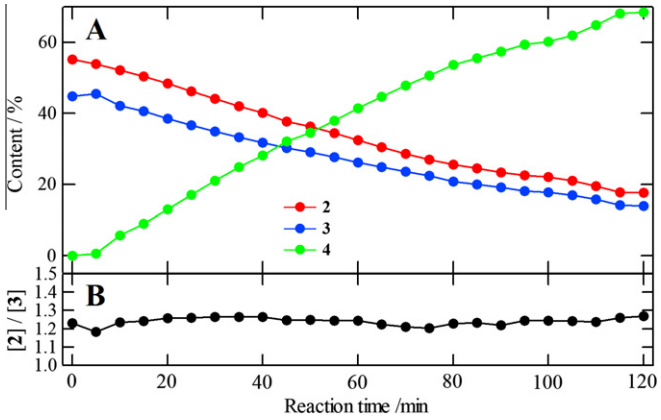


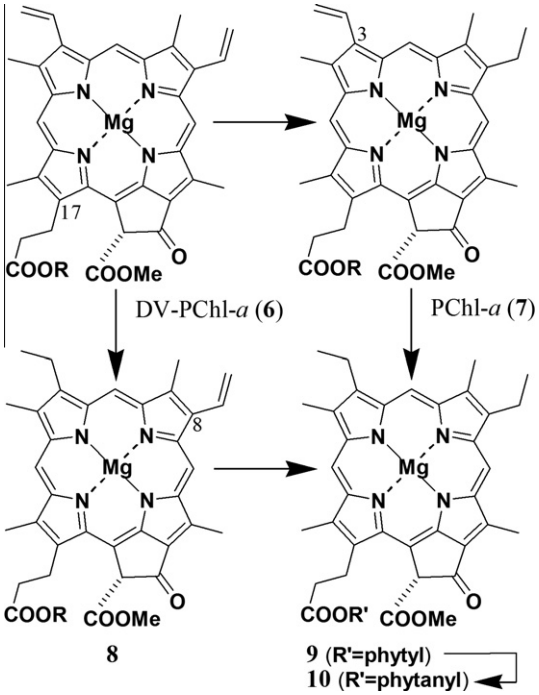
Figure 5. (A) Time-dependent contents of chlorophyll-*a* (Chl-*a*, **2**), 8-vinyl-mesochlorophyll-*a* (**3**) and mesochlorophyll-*a* (**4**) during the hydrogenation of a 1.2:1 mixture of **2** and **3** by the catalytic action of Rh/Al_2O_3 in acetone, determined from HPLC analysis using their relative extinction coefficients at 660 nm (see Section 4.2). (B) Time-dependent ratio of **2** over **3** during the above hydrogenation.

2.3. Hydrogenation of divinyl-protochlorophyll-*a*

Under the same conditions as for the reduction of chlorophylls (vide supra), divinyl-protochlorophyll-*a* (DV-PChl-*a*, **6**) was hydrogenated to give protochlorophyll-*a* (PChl-*a*, **7**), 8-vinyl-mesoprotochlorophyll-*a* (**8**) and mesoprotochlorophyll-*a* (**9**) without reduction of the C7=C8 and C17=C18 double bonds as shown in Scheme 3. The ^{1}H NMR spectrum of the reaction mixture depicts that the amount of 3-vinyl-porphyrin **7** was nearly equal to that of 8-vinyl-porphyrin **8** (see Figure 6). The observation in porphyrin π-systems is different from that in a hydrogenated mixture of DV-Chl-*a* (**1**) possessing a chlorin π-system where 1.2-fold less 3-vinyl-chlorin **2** was produced than 8-vinyl-chlorin **3**. The 3-vinyl group of **6** has the same reactivity for the present hydrogenation as the 8-vinyl group.

2.4. Biosynthetic reduction of divinyl-(proto)chlorophyllide-*a*

In vivo, divinyl-(proto)chlorophyllide-*a* [DV-(P)Chl-a_H] is reduced to give exclusively (proto)chlorophyllide-*a* [(P)Chl-a_H].[3–5,8]



Scheme 3. Stepwise hydrogenation of divinyl-protochlorophyll-*a* (DV-PChl-*a*, **6**) to mesoprotochlorophylls-*a* **9**/**10** through monovinyl-porphyrins **7** and **8**: **R** = **phytyl**.

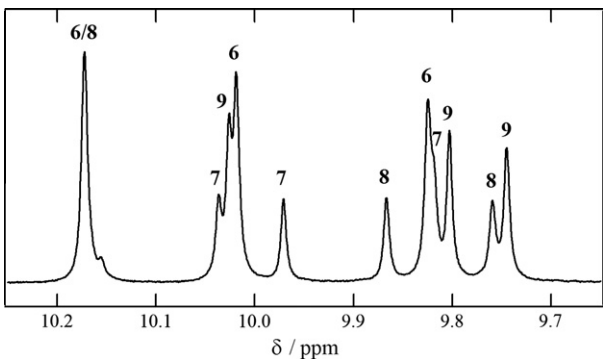


Figure 6. ^{1}H NMR spectrum of a hydrogenated mixture of divinyl-protochlorophyll-*a* (DV-PChl-*a*, **6**) containing **6**–**9** in 1% pyridine-d_5/$CDCl_3$, at the region giving *meso*-protons: δ(10-H) > δ(5-H) > δ(20-H) in each PChl.

Since the phytyl ester at the 17-propionate residue is unconjugated with the chlorin or porphyrin π-system, it cannot affect the reactivity of vinyl groups at the peripheral position of cyclic tetrapyrrole π-skeletons. Slightly selective mono-hydrogenation of DV-Chl-*a* (**1**) as well as non-selective hydrogenation of DV-PChl-*a* (**6**) in the present in vitro reduction largely differ from the completely selective in vivo reduction. Although the biosynthetic reduction by NADPH is different in its mechanism from the present catalytic hydrogenation, the predominant reduction of the 8-vinyl group in the enzymatic reduction could not be explained by the inherent reactivity of 3- and 8-vinyl moieties: the reactivity of 8-CH=CH_2 was comparable to (slightly more than or almost the same as) that of 3-CH=CH_2. The reduction enzyme, divinyl reductase, recognizes the 8-vinyl group as the reactive site at its reaction pocket to produce only the 8-ethylated compound, not the 3-ethylated product. The similarly reactive 3-vinyl group remains in the reduction and is modified in the later stage of biosynthesis of Chl-*d* (to 3-CHO), BChls-*a*/*b* (to 3-COMe) and BChls-*c*/*d*/*e* [to 3-CH(OH)Me].[3]

3. Concluding remarks

The 3- and 8-vinyl groups of DV-Chl-*a* (**1**) were hydrogenated stepwise by a catalytic amount of Rh/Al_2O_3 in acetone to give a mixture of the mono-reduced products, 3-vinyl-8-ethyl-chlorin **2** (Chl-*a*) and 3-ethyl-8-vinyl-chlorin **3**, followed by the doubly reduced 3,8-diethyl-chlorin **4**. After the two vinyl groups of phytylated **1** were reduced completely, the sterically crowded, three-substituted and inner double bond of the phytyl group of the resulting **4** was hydrogenated by a large amount of the catalyst. The same sequential hydrogenation was observed in the 17,18-dehydrogenated analog of chlorin **1**, DV-PChl-*a* (**6**) possessing a porphyrin π-skeleton.

The 3-vinyl group of **1** was hydrogenated 1.2 times more rapidly than the 8-vinyl group and the 3-CH=CH_2 of **6** was reduced at the same rate as the 8-CH=CH_2.[11] The small and no regioselectivities are in sharp contrast to the exclusive reduction of the 8-vinyl group in free carboxylic acid forms (17^2-COOH) of **1** and **6**, (P)Chl-a_H, during the biosynthetic pathways of most (B)Chls. Considering the similar reactivity of the vinyl groups, divinyl reductases recognize the 8-vinyl group as the reactive site perfectly.

8-Vinyl-chlorin **3** was separated from the isomeric 3-vinyl-chlorin **2** under the present HPLC conditions and fully characterized by visible, ^{1}H NMR and mass spectroscopies. A substitution effect was clearly observed in visible absorption bands (Qy and Soret maxima)[12] and chemical shifts of proton resonance peaks at the peripheral positions. Compound **3** prepared for the first time is the regioisomer of Chl-*a*, while Chl-*a*′ is known as the stereoisomer (13^2-epimer) of Chl-*a*.[2] The full identification of **3** would be useful for the detection of novel chlorophylls in natural phototrophs as well as artificial mutants.

4. Experimental

4.1. General

Visible absorption spectra were measured with a Hitachi U-3500 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a JEOL ECA-600 spectrometer (600 MHz); chloroform (δ = 7.26 ppm) was used as an internal reference. Mass spectra by ESI and atmospheric pressure chemical ionization (APCI) were measured on a Shimadzu LCMS-2010EV instrument and time-of-flight (TOF) mass data was obtained using direct laser desorption/ionization by a Shimadzu AXIMA-CFR plus spectrometer. Normal- and reverse-phase HPLC were performed on a packed silica gel (Cosmosil 5SL-II, Nacalai Tesque) and octadecylsilylated silica gel column (Cosmosil $5C_{18}$-AR-II or Inertsil ODS-P, GL Sciences), respectively, with a Shimadzu LC-10ADvp pump as well as SPD-M10Avp photodiode-array and/or LCMS-2010EV quadrupolar mass (ESI) detector. Flash column chromatography (FCC) was carried out on silica gel (Wakogel C-300, Wako).

DV-Chl-*a* (**1**) and Chl-*a* (**2**) were extracted from a cultured Δslr1923 mutant of *Synechocystis* sp. PCC6803[13] and commercially available *Spirulina geitleri*,[14] respectively, according to reported procedures. Their pure samples were obtained after FCC (acetone:dichloromethane = 1:9) and successive normal-phase HPLC (5SL-II 10 $\phi \times$ 250 mm, acetone:hexane = 2:8, 2 ml/min). Protochlorophylls-*a* were prepared by oxidation of the corresponding chlorophylls-*a* by DDQ in acetone as reported earlier:[13] see also Supplementary data. All the synthetic procedures were done in the dark. Solvents for HPLC and visible absorption spectroscopy were purchased from Nacalai Tesque as reagents prepared specially for HPLC and used without further purification.

4.2. Hydrogenation of chlorophylls-*a*

To an acetone solution (10 ml) of a (proto)chlorophyll sample (1 mmol) was added 5% rhodium on alumina (2 mg, Aldrich) and the mixture was stirred at room temperature under hydrogen atmosphere for an appropriate time. After filtration, the solvent was evaporated and the residue was analyzed by ^{1}H NMR and reverse-phase HPLC techniques. Relative extinction coefficients (εs) of chlorophylls at 660 nm were determined from the comparison of the NMR spectra with HPL chromatograms: ε(**1**)/ε(**2**)/ε(**3**)/ε(**4**) = 1.50/1.57/1.25/1 in water/methanol/acetonitrile = 1/19/20.

Analytically pure 8-vinyl-mesochlorophyll-*a* (**3**) was obtained after separation from the reaction mixture by reverse-phase HPLC ($5C_{18}$-AR-II 10 $\phi \times$ 250 mm, methanol:acetonitrile = 1:1, 3 ml/min, eluted at ca. 40 min). Mesochlorophylls-*a* (**4**/**5**) possessing phytyl and phytanyl esters were produced after 2-h hydrogenation of **2** by a catalytic and large amount of the catalyst, respectively, and purified by HPLC (5SL-II 10 $\phi \times$ 250 mm, acetone:hexane = 2:8, 2 ml/min, eluted at ca. 20 min).

4.3. Spectral data

4.3.1. Divinyl-chlorophyll-*a* (DV-Chl-*a*, 1)

VIS (acetone) λ_{max} = 662 (relative intensity, 0.73), 617 (0.12), 585 (0.06), 438 (1), 419 nm (0.65); ^{1}H NMR (1% pyridine-d_5/$CDCl_3$) δ = 9.72 (1H, s, 10-H), 9.37 (1H, s, 5-H), 8.30 (1H, s, 20-H), 8.02, 8.00 (each 1H, dd, *J* = 12, 18 Hz, 3-, 8-CH), 6.21, 6.16 (each 1H, dd, *J* = 1, 18 Hz, 3^1-, 8^1-CH *trans* to 3-, 8-C–H), 6.20 (1H, s, 13^1-CH), 6.02, 5.97 (each 1H, dd, *J* = 1, 12 Hz, 3^1-, 8^1-CH *cis* to 3-, 8-C–H), 5.13 (1H, t, *J* = 7 Hz, P1-CH), 4.44, 4.42 (each 1H, dd, *J* = 7, 12 Hz, 17^2-$COOCH_2$), 4.37 (1H, dq, *J* = 2, 7 Hz, 18-H), 4.08 (1H, m, 17-H), 3.84 (3H, s, 13^2-$COOCH_3$), 3.64 (3H, s, 12-CH_3), 3.38 (3H, s, 7-CH_3), 3.30 (3H, s, 2-CH_3), 2.53–2.44, 2.35–2.25, 1.92–1.80 (1H+2H+3H, m, 17-, 17^1-, P3-CH_2), 1.63 (3H, d, *J* = 7 Hz, 18-CH_3), 1.57 (3H, s, P3-CH_3), 1.48–0.90 (19H, m, P4-, P5-, P7-, P8-, P9-, P11-, P12-, P13-CH_2, P6-, P10-, P14-CH), 0.83 (6H, d, *J* = 7 Hz, P15-$(CH_3)_2$), 0.79, 0.77 (each 3H, d, *J* = 7 Hz, P7-, P11-CH_3); MS (ESI) found: *m*/*z* 891.7 and 612.2, calcd for $C_{55}H_{71}N_4O_5Mg$: MH^+, 891.5 and $C_{35}H_{32}N_4O_5Mg$: $[MH-phytyl]^+$, 612.2.

4.3.2. Chlorophyll-*a* (Chl-*a*, 2)

VIS (acetone) λ_{max} = 662 (relative intensity, 0.81), 616 (0.14), 579 (0.08), 430 (1), 412 nm (0.71); ^{1}H NMR (1% pyridine-d_5/$CDCl_3$) δ = 9.55 (1H, s, 10-H), 9.29 (1H, s, 5-H), 8.28 (1H, s, 20-H), 8.00 (1H, dd, *J* = 12, 18 Hz, 3-CH), 6.20 (1H, s, 13^1-CH), 6.19 (1H, dd, *J* = 1, 18 Hz, 3^1-CH *trans* to 3-C–H), 6.00 (1H, dd, *J* = 1, 12 Hz, 3^1-CH *cis* to 3-C–H), 5.13 (1H, t, *J* = 7 Hz, P1-CH), 4.43, 4.41 (each 1H, dd, *J* = 7, 12 Hz, 17^2-$COOCH_2$), 4.35 (1H, dq, *J* = 2, 7 Hz, 18-H), 4.07

(1H, m, 17-H), 3.83 (3H, s, 13^2-$COOCH_3$), 3.73 (2H, q, J = 7 Hz, 8-CH_2), 3.65 (3H, s, 12-CH_3), 3.30 (3H, s, 2-CH_3), 3.24 (3H, s, 7-CH_3), 2.50–2.44, 2.35–2.27, 1.94–1.85 (1H+2H+3H, m, 17-, 17^1-, P3-CH_2), 1.68 (3H, t, J = 7 Hz, 8^1-CH_3), 1.62 (3H, d, J = 7 Hz, 18-CH_3), 1.57 (3H, s, P3-CH_3), 1.49–0.95 (19H, m, P4-, P5-, P7-, P8-, P9-, P11-, P12-, P13-CH_2, P6-, P10-, P14-CH), 0.84 (6H, d, J = 7 Hz, P15-$(CH_3)_2$), 0.80, 0.77 (each 3H, d, J = 7 Hz, P7-, P11-CH_3); MS (ESI) found: m/z 893.7 and 614.3, calcd for $C_{55}H_{73}N_4O_5Mg$: MH^+, 893.5 and $C_{35}H_{34}N_4O_5Mg$: $[MH-phytyl]^+$, 614.2.

4.3.3. 8-Vinyl-mesochlorophyll-*a* (3)

VIS (acetone) λ_{max} = 652 (relative intensity, 0.79), 611 (0.13), 581 (0.07), 433 (1), 414 nm (0.71); ^{1}H NMR (1% pyridine-d_5/$CDCl_3$) δ = 9.68 (1H, s, 10-H), 9.15 (1H, s, 5-H), 8.20 (1H, s, 20-H), 8.20 (1H, dd, J = 12, 18 Hz, 8-CH), 6.18 (1H, s, 13^1-CH), 6.16 (1H, dd, J = 1, 18 Hz, 8^1-CH *trans* to 8-C–H), 5.96 (1H, dd, J = 1, 12 Hz, 8^1-CH *cis* to 8-C–H), 5.13 (1H, t, J = 7 Hz, P1-CH), 4.44, 4.41 (each 1H, dd, J = 7, 12 Hz, 17^2-$COOCH_2$), 4.33 (1H, dq, J = 2, 7 Hz, 18-H), 4.06 (1H, m, 17-H), 3.84 (3H, s, 13^2-$COOCH_3$), 3.71 (2H, q, J = 7 Hz, 3-CH_2), 3.63 (3H, s, 12-CH_3), 3.38 (3H, s, 7-CH_3), 3.16 (3H, s, 2-CH_3), 2.48–2.42, 2.33–2.25, 1.93–1.86 (1H+2H+3H, m, 17-, 17^1-, P3-CH_2), 1.68 (3H, t, J = 7 Hz, 3^1-CH_3), 1.62 (3H, d, J = 7 Hz, 18-CH_3), 1.57 (3H, s, P3-CH_3), 1.49–0.95 (19H, m, P4-, P5-, P7-, P8-, P9-, P11-, P12-, P13-CH_2, P6-, P10-, P14-CH), 0.85 (6H, d, J = 7 Hz, P15-$(CH_3)_2$), 0.80, 0.78 (each 3H, d, J = 7 Hz, P7-, P11-CH_3); MS (ESI) found: m/z 893.7 and 614.2, calcd for $C_{55}H_{73}N_4O_5Mg$: MH^+, 893.5 and $C_{35}H_{34}N_4O_5Mg$: $[MH-phytyl]^+$, 614.2.

4.3.4. Mesochlorophyll-*a* (4)

VIS (acetone) λ_{max} = 650 (relative intensity, 0.88), 608 (0.15), 573 (0.10), 425 (1), 406 nm (0.80); ^{1}H NMR (1% pyridine-d_5/$CDCl_3$) δ = 9.51 (1H, s, 10-H), 9.08 (1H, s, 5-H), 8.18 (1H, s, 20-H), 6.18 (1H, s, 13^1-CH), 5.15 (1H, t, J = 7 Hz, P1-CH), 4.43, 4.42 (each 1H, dd, J = 7, 12 Hz, 17^2-$COOCH_2$), 4.31 (1H, dq, J = 2, 7 Hz, 18-H), 4.05 (1H, m, 17-H), 3.83 (3H, s, 13^2-$COOCH_3$), 3.72, 3.70 (each 2H, q, J = 7 Hz, 3-, 8-CH_2), 3.64 (3H, s, 12-CH_3), 3.24 (3H, s, 7-CH_3), 3.15 (3H, s, 2-CH_3), 2.48–2.42, 2.33–2.26, 1.93–1.84 (1H+2H+3H, m, 17-, 17^1-, P3-CH_2), 1.68 (6H, t, J = 7 Hz, 3^1-, 8^1-CH_3), 1.61 (3H, d, J = 7 Hz, 18-CH_3), 1.58 (3H, s, P3-CH_3), 1.49–0.96 (19H, m, P4-, P5-, P7-, P8-, P9-, P11-, P12-, P13-CH_2, P6-, P10-, P14-CH), 0.84 (6H, d, J = 7 Hz, P15-$(CH_3)_2$), 0.80, 0.78 (each 3H, d, J = 7 Hz, P7-, P11-CH_3); MS (ESI) found: m/z 895.7 and 616.3, calcd for $C_{55}H_{75}N_4O_5Mg$: MH^+, 895.6 and $C_{35}H_{36}N_4O_5Mg$: $[MH-phytyl]^+$, 616.3.

4.3.5. Mesochlorophyll-*a* phytanyl ester (5)

VIS (acetone) λ_{max} = 650 (relative intensity, 0.88), 608 (0.15), 573 (0.10), 425 (1), 406 nm (0.80); ^{1}H NMR (1% pyridine-d_5/$CDCl_3$) δ = 9.51 (1H, s, 10-H), 9.08 (1H, s, 5-H), 8.18 (1H, s, 20-H), 6.17 (1H, s, 13^1-CH), 4.32 (1H, dq, J = 2, 7 Hz, 18-H), 4.05 (1H, m, 17-H), 3.92 (2H, m, 17^2-$COOCH_2$), 3.83 (3H, s, 13^2-$COOCH_3$), 3.72, 3.70 (each 2H, q, J = 7 Hz, 3-, 8-CH_2), 3.63 (3H, s, 12-CH_3), 3.24 (3H, s, 7-CH_3), 3.15 (3H, s, 2-CH_3), 2.48–2.42, 2.33–2.26, 1.93–1.90 (1H+2H+1H, m, 17-, 17^1-CH_2), 1.68 (6H, t, J = 7 Hz, 3^1-, 8^1-CH_3), 1.62 (3H, d, J = 7 Hz, 18-CH_3), 1.49–0.96 (24H, m, P1-, P3-, P4-, P5-, P7-, P8-, P9-, P11-, P12-, P13-CH_2, P2-, P6-, P10-, P14-CH), 0.84 (6H, d, J = 7 Hz, P15-$(CH_3)_2$), 0.80, 0.78 (3H+6H, d, J = 7 Hz, P3-, P7-, P11-CH_3); MS (TOF) found: m/z 896.6, calcd for $C_{55}H_{76}N_4O_5Mg$: M^+, 896.6.

Acknowledgments

We thank Prof. Ayumi Tanaka of Hokkaido University for giving us a Δslr1923 mutant of *Synechocystis* sp. PCC6803. This work was partially supported by a Grant-in-Aid for Scientific Research (A) (No. 22245030) from the Japan Society for the Promotion of Science (JSPS).

Supplementary data

Supplementary data (spectral data of protochlorophylls) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.11.056.

References and notes

1. Kräutler, B. *Photochem. Photobiol. Sci.* **2008**, *7*, 1114.
2. Tamiaki, H.; Shibata, R.; Mizoguchi, T. *Photochem. Photobiol.* **2007**, *83*, 152.
3. Porra, R. J. *Photochem. Photobiol.* **1997**, *65*, 492; Chew, A. G. M.; Bryant, D. A. *Annu. Rev. Microbiol.* **2007**, *61*, 113; Masuda, T.; Fujita, Y. *Photochem. Photobiol. Sci.* **2008**, *7*, 1131.
4. Nagata, N.; Tanaka, R.; Satoh, S.; Tanaka, A. *Plant Cell* **2005**, *17*, 233; Wang, P.; Gao, J.; Wan, C.; Zhang, F.; Xu, Z.; Huang, X.; Sun, X.; Deng, X. *Plant Physiol.* **2010**, *153*, 994 and references cited therein.
5. Kolossov, V. L.; Rebeiz, C. A. In *The Chloroplast: Basic and Applications*; Rebeiz, C. A., Benning, C., Bohnert, H. J., Daniell, H., Hoober, J. K., Lichtenthaler, H. K., Portis, A. R., Tripathy, B. C., Eds.; Springer: Dordrecht, The Netherlands, 2010; pp 25–38. Chapter 2.
6. Heyes, D. J.; Hunter, C. N. *Trends Biochem. Sci.* **2005**, *30*, 642; Muraki, N.; Nomata, J.; Ebata, K.; Mizoguchi, T.; Shiba, T.; Tamiaki, H.; Kurisu, G.; Fujita, Y. *Nature* **2010**, *465*, 110.
7. Mizoguchi, T.; Harada, J.; Tamiaki, H. *FEBS Lett.* **2006**, *580*, 6644; Tanaka, R.; Rothbart, M.; Oka, S.; Takabayashi, A.; Takahashi, K.; Shibata, M.; Myouga, F.; Motohashi, R.; Shinozaki, K.; Grimm, B.; Tanaka, A. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 16721.
8. Nagata, N.; Tanaka, R.; Tanaka, A. *Plant Cell Physiol.* **2007**, *48*, 1803.
9. Sherman, G.; Wang, S.-F. *J. Org. Chem.* **1966**, *31*, 1465; Belanger, F. C.; Duggan, J. X.; Rebeiz, C. A. *J. Biol. Chem.* **1982**, *257*, 4849.
10. Tamiaki, H.; Hamada, K.; Kunieda, M. *Tetrahedron* **2008**, *64*, 5721.
11. The small regioselective reduction of **1** might be explained by consideration of a chlorin π-conjugated system, similarly with the reduction of the corresponding carbonylated chlorins reported earlier.[10] The 3-vinyl group is attached on the largely delocalized 18π-system excluding the C7=C8 double bond and the 8-vinyl group is bonded with the relatively isolated C7=C8, giving that the conjugation of the 3-CH=CH_2 with the 18π-system would be weaker than that of the 8-CH=CH_2 with the C7=C8. Therefore, the 3-vinyl group is more isolated from the adjacent π-system than the 8-vinyl group, and the former is a more reactive vinyl moiety than the latter. A porphyrin π-conjugated skeleton is more symmetric than the chlorin and two different 18π-conjugated systems are possible in the porphyrin. One of π-systems includes the C7=C8 double bond. Both the 3- and 8-vinyl groups of **6** are connected with such largely delocalized 18π-systems, giving the same reactivity for the present hydrogenation.
12. Tamiaki, H.; Kunieda, M. In *Handbook of Porphyrin Science*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; World Scientific: Singapore, 2010; Vol. 11, pp 223–289. Chapter 51.
13. Mizoguchi, T.; Nagai, C.; Kunieda, M.; Kimura, Y.; Okamura, A.; Tamiaki, H. *Org. Biomol. Chem.* **2009**, *7*, 2120.
14. Tamiaki, H.; Takeuchi, S.; Tsudzuki, S.; Miyatake, T.; Tanikaga, R. *Tetrahedron* **1998**, *54*, 6699.