

Synthesis of chlorophyll-*a* derivatives methylated in the 3-vinyl group and their intrinsic site energy

Hitoshi Tamiaki^{a,*}, Kazuki Tsuji^{a,b}, Masaki Kuno^a, Yuki Kimura^{a,b}, Hiroaki Watanabe^a, Tomohiro Miyatake^b

^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



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ABSTRACT

Wittig reaction of methyl pyropheophorbide-*d* possessing the 3-formyl group gave readily methyl pyropheophorbides-*a* bearing a variety of 3-alkenyl groups as semi-synthetic models of chlorophyll-*a*. The 3-substituents rotated around the C3–C3¹ bond from the coplanar conformation with the chlorin π -system, moving the redmost visible absorption maxima to a shorter wavelength. The model experiments showed that natural chlorophyll-*a* carrying the 3-vinyl group would take a similar rotamer to control its intrinsic site energy.

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In the initial stage of natural photosynthesis, excitation energy transfer among various composite pigments occurs efficiently.¹ After light-absorption at peripheral antennas, the rapid energy flow to a reaction center through its core antenna is dependent on the site energies of the pigments (usually redmost absorption maxima) and their configuration (intermolecular distance and orientation) in the apparatus. The site energy is regulated by environmental factors (coordination, hydrogen bond, and π - π /CH- π /charge- π interaction) and pigment conformation (distortion of the π -skeleton and its π -conjugation with the peripheral substituents).² The latter controls the 'intrinsic' site energy and especially the substitution effect is important for determining the energy levels of chlorophyll (Chl) molecules possessing a relatively rigid cyclic tetrapyrrole whose π -plane can be deformed slightly.^{3,4}

In oxygenic phototrophs, Chl-*a* is the most abundant pigment, whose molecular structure is shown in the left drawing of Figure 1. The 3-vinyl group is directly attached with the chlorin π -system and the components are conjugated through the rotational C3–C3¹ single bond to affect the intrinsic site energy. The conformation of many Chl-*a* molecules in proteins are available from their crystallographic analysis data.⁵ For example, the dihedral angles θ^6 of C2(4)–C3–C3¹–C3² range from 1° to 52° in a

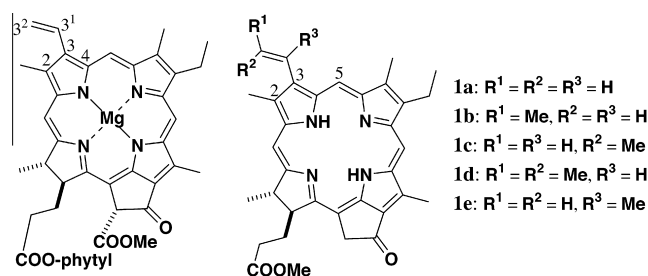


Figure 1. Molecular structures of naturally occurring chlorophyll-*a* (Chl-*a*, left) and its semi-synthetic (un)methylated models, methyl pyropheophorbides-*a* possessing a vinyl **1a**, *trans*-1-propenyl **1b**, *cis*-1-propenyl **1c**, 2-methyl-1-propenyl **1d**, or isopropenyl group **1e** at the 3-position (right).

cyanobacterial photosystem II (Protein Data Bank No. 3WU2, 1.9 Å resolution) which gave one of the highly resolved Chl-protein crystals.⁷ Such rotamers were theoretically investigated,⁸ but no experimental study has been available from manuscripts, to our best knowledge. Here we report synthesis of Chl-*a* derivatives **1** (see the right drawing of Fig. 1) possessing (un)methylated vinyl groups at the 3-position and compared their visible absorption bands in a diluted solution. The methylated vinyl groups would interact with the neighboring moieties in a molecule. The steric repulsion could rotate the 3-substituent from a coplanar

* Corresponding author. Fax: +81 77 561 3729.

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

conformation and induce its less π -conjugation with the chlorin system to shift hypsochromically the redmost maximum (= intrinsic site energy) of the synthetic models due to a small electronic effect of the additional methyl group(s).

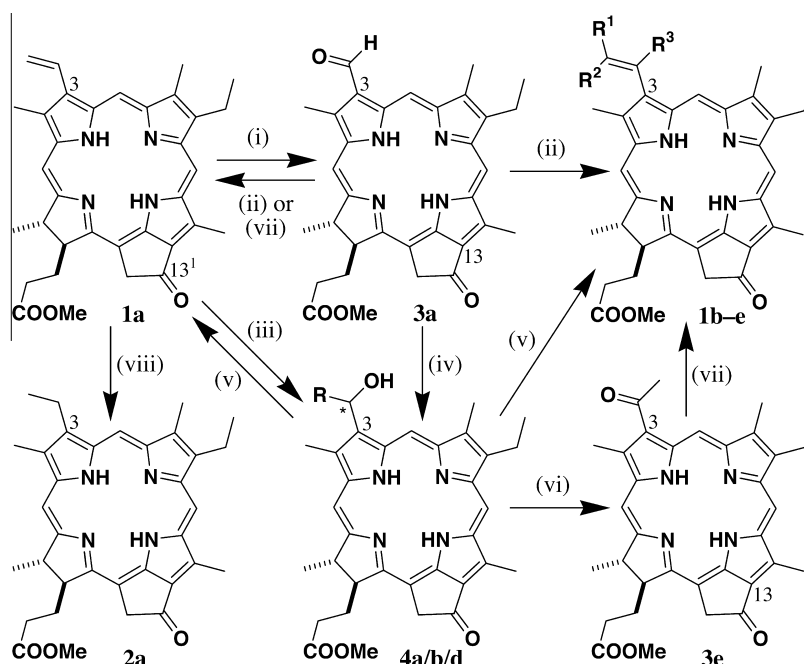
Methyl pyropheophorbide-*d* (**3a**) was prepared by modification of Chl-*a* extracted from commercially available *Spirulina* powder through oxidation of methyl pyropheophorbide-*a* (**1a**) [see step (i) of Scheme 1] or alternatively obtained from cultured cells of *Acaryochloris marina* producing Chl-*d* (the 3-formyl-substituted Chl-*a*).⁹ It was previously revealed that semi-synthetic 3-formylchlorin **3a** reacted with a variety of (*meta*)-stable Wittig reagents to readily give the corresponding olefins.^{10,11} In contrast, treatment of **3a** with one equivalent of the unstable Wittig reagent, methylenetriphenylphosphorane prepared by $\text{CH}_3\text{PPh}_3^+\text{I}^-$ and MeOLi was reported to afford a trace amount of its desired **1a**^{10,12} and even using an excess amount $\text{CH}_2=\text{PPh}_3$ (from $\text{CH}_3\text{PPh}_3^+\text{Br}^-$ and BuLi) led to the production of **1a** in a low yield (16%).^{13,14} The chemical yield was improved as follows.

When $\text{CH}_3\text{PPh}_3^+\text{I}^-$ was mixed with 1.5 equivalents of potassium *tert*-butoxide in dichloromethane at room temperature, the reaction mixture was quickly changed from white to brilliant yellow (or orange) color to give $\text{CH}_2=\text{PPh}_3$. Just after its preparation, a relatively small amount of **3a** was added and stirred for a few minutes to afford **1a** in an isolated yield of 46% by purification of column chromatography and successive recrystallization [see step (ii) of Scheme 1 and ESI]. From the reaction mixture no compound bearing 13- $\text{C}=\text{CH}_2$ was available, because the 13-keto carbonyl group was less reactive than the 3-formyl group. Such simple procedures are efficient for the regioselective Wittig reaction of **3a** to **1a**. Recently we reported similar methylenation of **3a** with a commercially available Tebbe reagent (61% isolated yield) [step (vii) of Scheme 1].¹⁶ The yield is larger than that by the present Wittig reaction, but the Tebbe reaction must be performed carefully. Since the Tebbe reagent was more reactive than the Wittig reagent, the former readily reacted with the 13- $\text{C}=\text{O}$ to give the 13¹-methylenated analog of **1a** after the prolonged reaction.

The aforementioned procedures of Wittig reaction were useful for preparation of 3-(2-methyl-1-propenyl)-chlorins **1b–d**. Ethyldienation of **3a** with $\text{MeCH}=\text{PPh}_3$ gave a 1:3 mixture of *cis*- and *trans*-isomers **1c/b** (16% isolated yield).¹⁷ The isomers were easily separated by a single run of isocratic HPLC (see ESI). Their characterization was performed by various spectroscopies: especially proton coupling constants of 3- $\text{CH}=\text{CH}$ were 11 and 16 Hz for *cis*-**1c** and *trans*-**1b**, respectively (ESI). The stereoselectivity is consistent with the reported ratio for benzylidenation of **3a**.¹⁰ Similarly as in acidic dehydration of methyl bacteriopheophorbide-*d* (**4a**) possessing a 1-hydroxyethyl group at the 3-position to **1a** [step (v) of Scheme 1],¹⁸ 3-(1-hydroxypropyl)chlorin **4b** prepared by Grignard reaction of **3a** with EtMgBr [step (iv)]¹⁹ was converted to thermodynamically stable *trans*-**1b** exclusively but no *cis*-**1c** was obtained. Simple isopropylidenation of **3a** with $\text{Me}_2\text{C}=\text{PPh}_3$ directly afforded **1d** (14%),¹⁷ while isopropylation of **3a** with *i*PrMgCl¹⁹ followed by acidic dehydration of the resulting secondary alcohol **4d** gave **1d** in the overall yield of less than a few% with more troublesome procedures.

Since the 3-acetyl group was known to be more reactive than the 13-keto-carbonyl group,²⁰ the methylenation of **3e** was examined using the above Wittig reaction procedures but no desired product was found in the reaction mixture. 3-Isopropenyl-chlorin **1e** was prepared by methylenation of **3e** with more reactive Tebbe reagent (15%).¹⁶ As the reference compound, 3-ethyl-chlorin **2a** was synthesized by catalytic hydrogenation of **1a** [step (viii) of Scheme 1].⁹

Methyl pyropheophorbides-*a* **1a–e** and **2a** were readily dissolved in dichloromethane to give intense visible absorption bands. The Qy and Soret bands of **1a–d** and **2a** at longer and shorter wavelengths, respectively, were sharp (see Fig. 2), indicating that they were monomeric in the diluted solution (ca. 10 μM). Isopropenylated chlorin **1e** showed also sharp but relatively broadened bands, whose spectral shape was apparently different from the others. This is ascribable to a distortion of the chlorin π -plane by the sterically demanding isopropenyl group.²¹



Scheme 1. Synthesis of methyl (di)methyl-pyropheophorbides-*a* **1b–e** from methyl pyropheophorbide-*a* (**1a**) through methyl pyropheophorbide-*d* (**3a**), methyl mesopyropheophorbide-*a* (**2a**) from **1a**, and **1a** from **3a**: (i) $\text{OsO}_4\text{--NaIO}_4/\text{THF--H}_2\text{O}$; (ii) $\text{R}^1\text{R}^2\text{CHPPh}_3^+\text{I}^-t\text{BuOK}/\text{CH}_2\text{Cl}_2$; (iii) HBr/AcOH , H_2O , $\text{CH}_2\text{N}_2/\text{Et}_2\text{O}$; (iv) $\text{MeMgI}/\text{Et}_2\text{O}$, EtMgBr/THF , or $i\text{PrMgCl}/\text{THF}$; (v) $p\text{-MeC}_6\text{H}_4\text{SO}_3\text{H--H}_2\text{O}/\text{PhMe}$ (90 °C); (vi) $\text{Pr}_4\text{NRuO}_4\text{--MeN}(\text{O})(\text{CH}_2\text{CH}_2)_2\text{O}/\text{CH}_2\text{Cl}_2$; (vii) $\text{Cp}_2\text{Ti}(\text{--Cl})(\text{--CH}_2\text{--})\text{AlMe}_2\text{--C}_5\text{H}_5\text{N}/\text{PhMe--THF}$ (−20 °C); (viii) $\text{H}_2\text{--Pd}/\text{C}/\text{THF--Me}_2\text{CO}$.

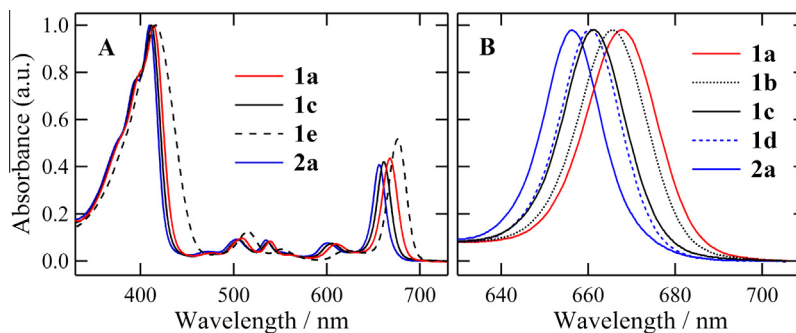


Figure 2. Visible absorption spectra of methyl pyropheophorbides-**a** **1a** (3-CH=CH₂), **1b** (*trans*-CH=CHMe), **1c** (*cis*-CH=CHMe), **1d** (CH=CMe₂), **1e** (CMe=CH₂), and **2a** (CH₂CH₃) in dichloromethane (ca. 10 μM). All the spectra are normalized by the intense maxima.

Therefore, the redmost Qy maxima $\lambda_{\max}(\text{Qy})$ of **1a–d** and **2a** were compared to clarify the conformational effect of their 3-substituents on the maxima. Their Qy maxima were blue-shifted in the order of **1a/b**, **1c/d**, and **2a**: $\lambda_{\max}(\text{Qy}) = 668/666$ (**1a/b**) > 661/660 (**1c/d**) > 656 nm (**2a**). The *cis*-methylation to 3-vinyl- and 3-*trans*-1-propenyl-chlorins as in **1a** → **1c** and **1b** → **1d** shifted the Qy maxima to a shorter wavelength by 6–7 nm. Such *cis*-methylated substituents are so bulky at around the 3-position that the planar moiety should interact with the neighboring 2-methyl group and 5-hydrogen atom to rotate from the coplanar with chlorin π -system to the perpendicular direction (see also the drawings of Graphic Abstract). The resulting lower π -conjugation would induce blue shifts of the Qy maxima.

The above *cis*-methylation led to high-field shifts of proton peaks δ of the 2-methyl group and 5-hydrogen atom in their ¹HNMR spectra: $\delta(2\text{-CH}_3) = 3.41/36$ (**1a/b**) → 3.21/21 ppm (**1c/d**) and $\delta(5\text{-H}) = 9.38/32$ (**1a/b**) → 9.16/14 ppm (**1c/d**) in CDCl₃ (see ESI). These protons of **1c/d** would be situated above the 3-CH=C π -plane and deshielded by the double bond to give high-field shifted δ -values. The ¹HNMR spectral analysis supported the aforementioned rotation of the 3-substituents in **1a/b** → **1c/d**.

Molecular modeling by MM+/PM3 calculation³ indicated a similar conformational change as mentioned above. In the energy-minimized structures, the dihedral angles θ^6 of C2(4)–C3–C3¹–C3² increased in the order of **1a/b** and **1c/d**: $\theta = 4.7/10.0$ (**1a/b**) < 60.6/62.6° (**1c/d**). A π – π orbital overlapping degree is dependent on $\cos^2 \theta$, which should be related to absorption maxima.²² Since 3-ethyl-chlorin **2a** lacked the π -conjugation between the 3-ethyl and chlorin moieties, the $\cos^2 \theta$ was proposed to be zero. The observed Qy maxima were found to be well correlated with the estimated $\cos^2 \theta$ (see Fig. 3),²³ indicating that **1a–d** would take such conformational rotamers. Therefore, the intrinsic site energy of Chl-*a*

can be controlled by the rotation of the C3–C3¹ bond, which was first induced from the experimental results.

The present procedures of the Wittig reaction were utilized for facile olefination of the 3-formyl group and would be applied for the selective transformation of CHO to C=CR¹R² at the other peripheral positions of chlorophyllous pigments.²⁴ Additionally, the resulting olefins **1b–d** could be hydrogenated as in **1a** → **2a** to give 3-propyl- and isobutyl-chlorins. Since such alkyl groups are found in the 8-substituent of bacteriochlorophylls-*c/d/e/f*,²⁵ the Wittig reaction of the 8-formyl group and the successive hydrogenation²⁶ would be useful for the preparation of their synthetic models.

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Supplementary data

Supplementary data (synthetic details of **1b–e**) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2016.05.008>.

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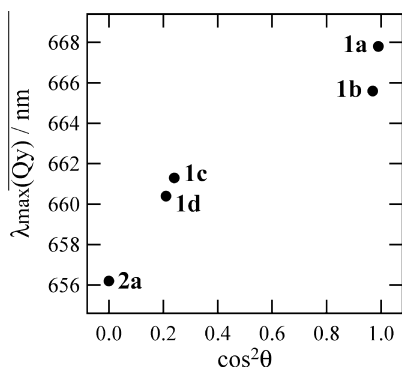


Figure 3. Dependence of observed Qy absorption maxima $\lambda_{\max}(\text{Qy})$ (CH₂Cl₂) on estimated dihedral angles θ (C2(4)–C3–C3¹–C3², MM+/PM3 calculation) of methyl pyropheophorbides-**a** **1a** (3-CH=CH₂), **1b** (*trans*-CH=CHMe), **1c** (*cis*-CH=CHMe), **1d** (CH=CMe₂), and **2a** (CH₂CH₃).

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