



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



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## 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-divinyl-chlorins 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.

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## 1. Introduction

Phototrophs have several pigments including cyclic tetrapyrrole chlorophylls (Chls), linear tetrapyrrole bilins, and polyene carotenoids.<sup>1,2</sup> 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-position (left drawing of Fig. 1) except Chl-*d* bearing the 3-formyl group in the limited cyanobacterial species, such as *Acaryochloris marina*.<sup>3–5</sup> 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* → divinyl(DV)-Chl-*a* (*Synechococcus/Prochlorococcus*),<sup>6,7</sup> Chl-*b* → DV-Chl-*b* (*Prochlorococcus* sp. NIES-2086),<sup>8</sup> Chl-*c*<sub>1</sub> → Chl-*c*<sub>2</sub> (*Chaetoceros calcitrans*),<sup>9</sup> and

monovinyl(MV)-Chl-*c*<sub>3</sub> → Chl-*c*<sub>3</sub> (*Emiliania huxleyi*),<sup>10</sup> 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.<sup>3,10,11</sup> 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.<sup>12</sup> 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*<sub>1/c</sub><sub>2/d/f</sub> is a methyl group, while a formyl or methoxycarbonyl group is found in (DV-)Chl-*b* or (MV-)Chl-*c*<sub>3</sub>, respectively.<sup>3,4,10</sup> 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, pyridinium dichromate; 7V, 7-vinyl.

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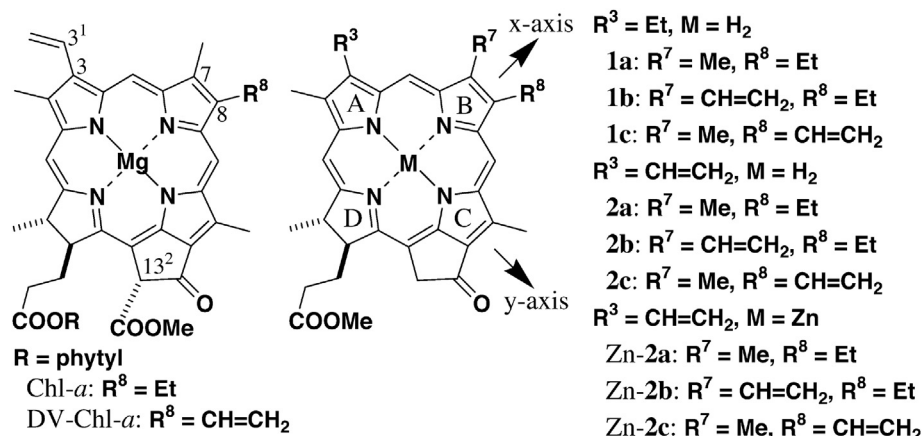


Fig. 1. Molecular structures of natural [divinyl(DV)]-chlorophylls(Chls)-*a* (left) and their synthetic analogs (right).

## 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.<sup>5,13</sup> The 3-vinyl group of **2a** was catalytically hydrogenated to give methyl mesopyropheophorbide-*a* (**1a**), as shown in step (i) of Scheme 1.<sup>13,14</sup> 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)].<sup>5,15</sup>

The 3-vinyl substituent in **2a** was hydrated to a 3<sup>1</sup>-epimeric mixture of the corresponding secondary alcohol, methyl 8-ethyl-12-methyl-bacteriopheophorbide-*d* ([E,M]BPhe-*d*<sub>M</sub>) [step (iv) of Scheme 1]. The carbinol was also obtained from chemical alteration of extracted bacteriochlorophyll(BChl)-*a* or an isolated BChl-*d* homolog.<sup>13</sup> Similarly to the synthesis of **1d** from **1a**, triol **2d** was obtained by the oxidation of [E,M]BPhe-*d*<sub>M</sub><sup>15</sup> [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.<sup>16</sup> The crude alcoholic products were directly heated in 1,2-dichlorobenzene 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.<sup>17</sup> The one-pot simple conversion of **2d** to **2c** proceeded by refluxing the mixed solvents (1:9 CH<sub>2</sub>Cl<sub>2</sub>–C<sub>6</sub>H<sub>6</sub>) with the same acid<sup>18</sup> but the isolated yield was lower (9%) than the above. Moreover, the Wittig reaction (vide infra)<sup>19–23</sup> of 3-formyl-8-vinyl-chlorin, methyl 8-vinyl-pyropheophorbide-*d*,<sup>15</sup> with CH<sub>2</sub>=PPh<sub>3</sub> 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<sup>9,24</sup> based on the same procedures as in the aforementioned transformation of Chl-*a* to **2a**.

Chl-*b* from spinach was altered to methyl 7<sup>1</sup>-hydroxy-pyropheophorbide-*a* (**2f**)<sup>5</sup> 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<sup>5,23</sup> to afford 3,7-divinyl-chlorin **2b** [42%, step (vii)]. The new compound was identified by its <sup>1</sup>H NMR, mass, and visible spectra (section 4.2.2). According to previously reported procedures,<sup>5</sup> 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 3,7-divinyl-chlorin **2b** gave a mixture of 3-vinyl-chlorin **2a** and 7-vinyl-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.<sup>25</sup> It is noteworthy that the enzymatic reduction of divinyl-chlorophyllide-*a* (the dephytylated derivative of DV-Chl-*a*) proceeds regioselectively at the 8-vinyl group to give solely the product monovinylated at the 3-position, chlorophyllide-*a*.<sup>12</sup>

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 13<sup>2</sup>-methoxycarbonyl group and are substituted at the central metal (Mg → Zn) and esterifying group (phytyl → 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 μM) 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 Q<sub>x</sub> bands, and the bands in the yellow to red region are Q<sub>y</sub> bands. All three visible bands are composed of the main band at a longer wavelength and the minor bands at shorter wavelengths. The Q<sub>x</sub> and Q<sub>y</sub> 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 Q<sub>y</sub> bands (1 nm) and red-shift of the Soret band by 10 nm. The substitution largely affected the shape and positions of the Q<sub>x</sub> bands. The relative Q<sub>x</sub>(0,0) band intensity of **1b** halved in comparison with that of **1a**. The Q<sub>x</sub>(0,0)/

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