



C3- and C13-substituent effects on electronic absorption spectra of linear tetrapyrroles produced by photooxidation of zinc chlorophyll derivatives

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ABSTRACT

Zinc chlorophyll derivatives possessing various substituents at the 3- and 13-positions were cleaved at the C19–C20 bond upon irradiation of an oxygen-saturated dichloromethane solution with visible light. The resulting photooxidation products were zinc complexes of linear tetrapyrroles which showed longer wavelength absorption bands in the near-infrared region than the visible Qy bands of the corresponding cyclic tetrapyrroles as the starting materials. Electron-withdrawing C13-substituents of the linear tetrapyrroles shifted the longest absorption maxima bathochromically, as observed in the cyclic tetrapyrroles. In contrast, more electron-withdrawing C3-substituents of the linear tetrapyrroles gave more hypsochromically shifted redmost maxima, which was the reverse of the substitution effect of the cyclic tetrapyrroles.

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1. Introduction

Linear tetrapyrroles as well as cyclic tetrapyrroles including hemes and chlorophyll(Chl)s, have very important roles in nature. In photosynthesis, for example, phycocyanin (one of the linear tetrapyrroles) absorbs light at 500–600 nm effectively, where Chls-*a/b* as the other light-harvesting pigments absorb it less. Phytochromobilin (another linear tetrapyrrole) is essential in regulating various events, such as seed germination, chloroplast development, shade avoidance and flowering in higher plants [1]. Recently, it was reported that bilirubin possessing a linear tetrapyrrole skeleton and its analogs function as antioxidants [2,3]. To elucidate the *in vivo* reactions using linear tetrapyrroles at a molecular level, the related pigments were synthesized by various procedures [4]. One of these procedures is oxidative cleavage of cyclic tetrapyrroles, porphyrins and chlorins, at their *meso*-positions by irradiation of visible light [5] or treatment of oxidative reagents [6,7]. The photooxidation of chlorins has been researched for several decades, because the photocleavage might occur in Chl degradation [8] and also the products are useful as model compounds of Chl catabolites [9,10].

As shown in Scheme 1, zinc complex of methyl pyropheophorbide-*a* (**1b**), one of the Chl-*a* derivatives, was reported to be regioselectively cleaved at the C19–C20 bond on irradiation with visible light in the presence of air to give zinc complex of linear tetrapyrrole (**1c**), while the corresponding cadmium complex was opened at the C4–C5 [11]. Another report

[12] indicated that photooxidation of a metal-free 20-methyl-chlorin (methyl 20-methyl-mesopyropheophorbide-*a*) provided its C1–C20 cleaved compound (see Scheme 2).

It is known that electronic absorption spectra of Chls and their derivatives are dependent on the peripheral substituents conjugated with their π -systems [13,14]. In naturally occurring Chls, Chl-*a*, Chl-*d* and bacteriochlorophyll-*d*, which have vinyl, formyl and 1-hydroxyethyl group at the 3-position, respectively (Fig. 1), give their redmost Qy peaks in electronic absorption spectra at 661, 686 and 650 nm in diethyl ether [14–16]. Chemical modification into such peripheral substituents was achieved for Chl derivatives and their Qy maxima have been widely regulated [14]. We have reported systematic preparation of Chl derivatives possessing different C3- and C13-substituents which fine-tuned their Qy peak positions at 640–740 nm [17–19]: the larger electronegativity of such substituents on the *y*-axis (Scheme 3) shifted their Qy maxima to a longer wavelength. However, to our knowledge, there is no work elucidating the correlation between absorption spectra of linear tetrapyrroles and peripheral substituents conjugated with their π -systems.

In this study, we synthesize zinc complexes of linear tetrapyrroles by photooxidation of Chl-*a* derivatives possessing several C3- and C13-substituents on the *y*-axis, and discuss the substitution effects on their optical properties.

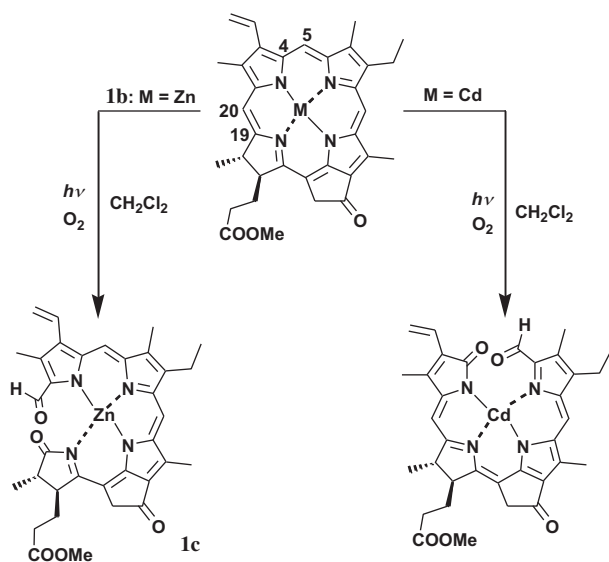
2. Experimental

2.1. General

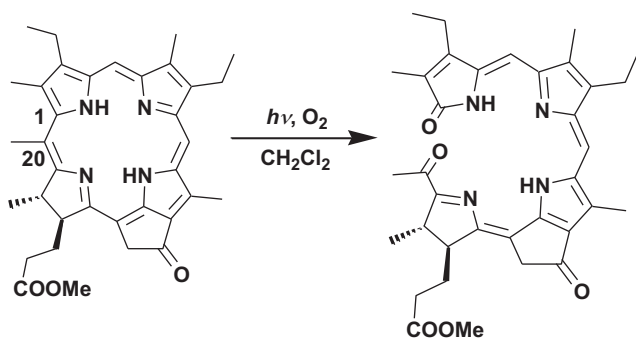
Electronic absorption and fluorescence emission spectra were measured in dichloromethane on Hitachi U-3500 and F-4500

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Scheme 1. Photooxidation of zinc and cadmium complexes of methyl pyropheophorbide-*a*.



Scheme 2. Photooxidation of methyl 20-methyl-mesopyropheophorbide-*a*.

spectrophotometer, respectively. ^1H NMR spectra in CDCl_3 were recorded with a JEOL ECA-600 spectrometer; chemical shifts (δ s) are expressed in parts per million relative to CHCl_3 (7.26 ppm) as an internal reference. Laser desorption ionization time-of-flight (TOF) mass spectroscopy (MS) was performed with a Shimadzu AXIMA-CFRplus spectrometer. Flash column and gel filtration chromatography was done with silica gel 60 (Merck) and sephacryl

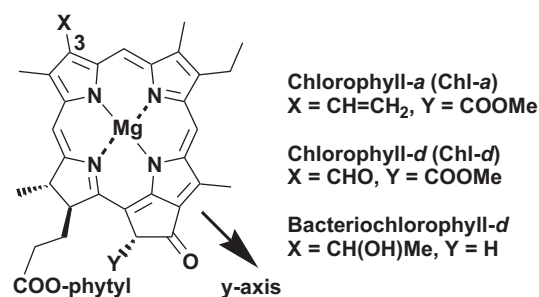


Fig. 1. Chemical structures of some natural (bacterio)chlorophylls.

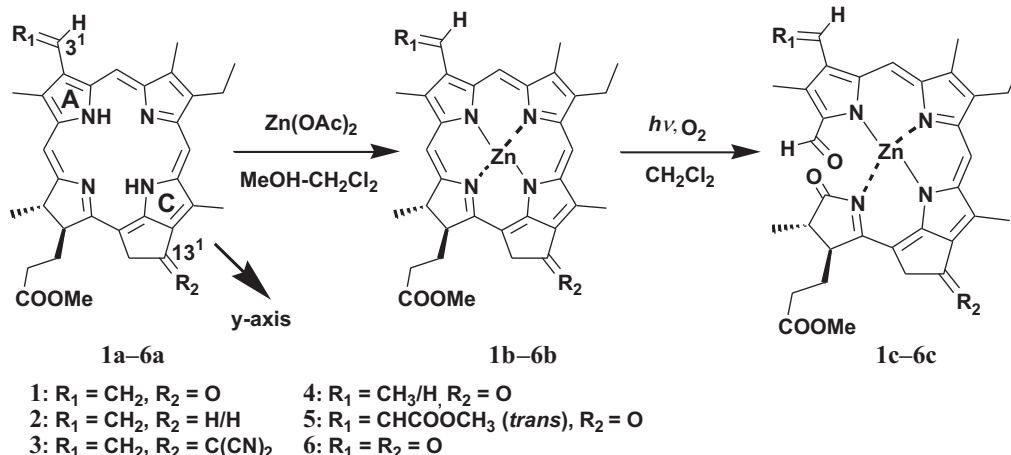
S-100 HR gel (GE Healthcare), respectively. A silica gel 60 F₂₅₄ plate (Merck) was used for TLC analysis. Extra pure and specially prepared (for spectral analysis) reagent grade dichloromethane (Nacalai Tesque) was used without further purification for photoreaction and spectral analysis, respectively. Chl derivatives **1a** [20,21], **2a** [22], **3a** [19], **4a** [20], **5a** [17] and **6a** [23] were synthesized according to reported procedures.

2.2. Zinc metallation of chlorophyll derivatives

A methanol solution saturated with $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ (1 mL) was added to a dichloromethane (10 mL) solution of metal-free chlorins **1a–6a** (10 mg) and stirred at room temperature under nitrogen [22]. After visible absorption and TLC analyses showed the disappearance of the starting metal-free chlorin, the reaction mixture was poured into aq. 4% NaHCO_3 , and stirred for 10 min. White precipitates produced were removed by filtration and the filtrate was dried over Na_2SO_4 and evaporated. The resulting residue was recrystallized from dichloromethane and hexane to give the corresponding zinc complex. Additional recrystallization afforded an analytically pure sample, which was used for spectroscopic analyses. Their visible absorption and TOF-MS spectral data are described in supporting information.

2.3. Photooxidation of zinc chlorophyll derivatives

A diluted solution (ca. 5×10^{-5} M) of a zinc chlorin in dichloromethane was irradiated by two 250 W halogen lamps (ELMO slide projector OMNI 253 AF, 350–700 nm) at a distance of 15 cm from the flat surface of a reaction flask with bubbling oxygen gas for 1 h [11]. The reaction mixture was concentrated to one-fifth of its original volume and twice the volume of hexane was added,



Scheme 3. Synthesis of zinc complexes of linear tetrapyrroles **1c–6c** by photooxidation of **1b–6b** prepared by zinc-metallation of **1a–6a**.

followed by evaporation of all the solvents to dryness. The residue was purified by silica gel (diethyl ether and dichloromethane) and sephacryl gel (hexane and dichloromethane) column chromatography. Their electronic absorption, ^1H NMR and TOF-MS spectral data are described in supporting information.

2.4. Molecular modeling calculation

The program package HyperChem version 8.0 was used for all the molecular modeling calculations [24]. Semi-empirical quantum-chemical and molecular mechanics calculations were performed by PM3 and MM+ methods, respectively. In all the cases, the original parameters provided in the HyperChem package were employed. Maintaining the distribution of partial charges determined for PM3-optimized chlorins, the structures were energy minimized by MM+. Generally a stop criterion of gradient <0.01 was employed. PM3 calculation was done again on the basis of the structure given by the MM+ calculation. PM3/MM+ calculations were continued until the energy and the structure were unchanged to give the optimal structure. Finally, electronic absorption maxima of each structurally optimized compound were estimated by ZINDO/S.

3. Results and discussion

We synthesized Chl-*a* derivatives **1a–6a** possessing several C3- and C13-substituents on the *y*-axis according to reported procedures [17,19,22,23]. Methyl pyropheophorbide-*a* (**1a**, see the left drawing of Scheme 3) was easily prepared from naturally occurring Chl-*a* [20,21] and used as the starting material. 3-Vinyl-13¹-oxo-chlorin **1a** was modified at the 13¹-position to give 13¹-deoxy-chlorin **2a** and 13¹-dicyanomethylene-chlorin **3a** and also at the 3¹-position to afford 3-ethyl-chlorin **4a**, 3-*trans*-(methoxycarbonyl)ethenyl-chlorin **5a** and 3-formyl-chlorin **6a**. Metal-free chlorins **1a–6a** were converted to the corresponding zinc complexes **1b–6b** quantitatively by stirring with zinc acetate in methanol and dichloromethane [22]. Iturraspe and Gossauer [11] reported that the photoreaction of zinc methyl pyropheophorbide-*a* (**1b**) in an oxygen-saturated dichloromethane solution gave zinc methyl 19,20-dioxo-19,20-*seco*-pyropheophorbide *a* (**1c**) as an isolable cleaved product (see Scheme 1). Similar photooxidation of **1b–6b** was carried out by irradiation of their dilute dichloromethane solutions (ca. 5×10^{-5} M) with visible light for 1 h. After purification by silica gel and sephacryl gel column chromatography, zinc complexes of linear tetrapyrroles **1c–6c** (see the right drawing of Scheme 3) were obtained as oxidative ring-opened products and identified by measurements of their UV-Vis-NIR, ^1H NMR and mass spectra. Reverse-phase HPLC purification of the photooxidized products was ineffective due to their instability in protic solvents as eluents.

As well as free base chlorophyll derivatives **1a–3a**, zinc complex of methyl pyropheophorbide-*a* (**1b**) and its C13¹-modified derivatives **2b** and **3b** gave intense Soret and Qy bands in their absorption spectra (Fig. S1). Both the maxima moved to a longer wavelength in the order of **2a/b** \rightarrow **1a/b** \rightarrow **3a/b**. Typically, Fig. 2 shows that the removal of the 13¹-oxo group (**1b** \rightarrow **2b**) caused a blue shift of Qy peak (655 \rightarrow 624 nm), while the substitution of an oxo group with a dicyanomethylene group at the 13¹-position (**1b** \rightarrow **3b**) caused a red shift of Qy peak (655 \rightarrow 700 nm) (see also Table 1). The C19–C20 oxidative cleavage of cyclic tetrapyrroles **1b–3b** to linear tetrapyrroles **1c–3c** bathochromically shifted their redmost peaks (Figs. 2 and 3). It is noteworthy that **3c** absorbs near-infrared light in the region over 1100 nm. Similar to cyclic tetrapyrroles **1b–3b**, Fig. 3 shows that the reduction of the C13-substituent of linear tetrapyrroles (**1c** \rightarrow **2c**) caused blue shifts of the redmost

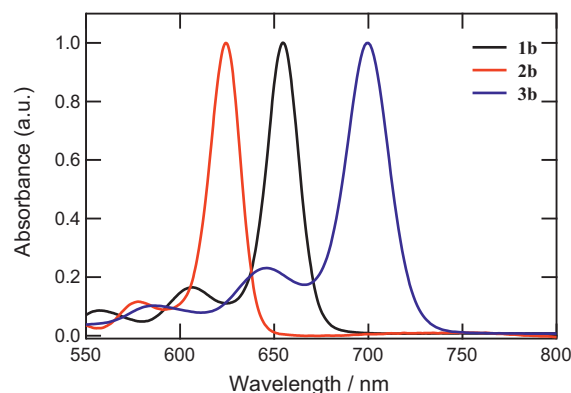


Fig. 2. Electronic absorption spectra (≥ 550 nm) of **1b–3b** in dichloromethane. Normalized at each Qy peak.

Table 1

Redmost electronic absorption maxima (λ_{max} /nm) of cyclic and linear tetrapyrroles, **1b–6b** and **1c–6c**.

Compound	R ₁ (C3 ¹)	R ₂ (C13 ¹)	λ_{max} /nm	
			Cyclic	Linear
2	CH ₂	H/H	624 (2b)	800 (2c)
1	CH ₂	O	655 (1b)	858 (1c)
3	CH ₂	C(CN) ₂	700 (3b)	919 (3c)
4	CH ₃ /H	O	644 (4b)	861 (4c)
1	CH ₂	O	655 (1b)	858 (1c)
5	CHCOOCH ₃ ^a	O	671 (5b)	851 (5c)
6	O	O	681 (6b)	823 (6c)

^a *Trans*-configuration.

peak and its lower wavelength shoulder (858 and 771 \rightarrow 800 and 723 nm), while the introduction of a dicyanomethylene group at the 13¹-position (**1c** \rightarrow **3c**) caused red shifts of the peak and shoulder (858 and 771 \rightarrow 919 and 830 nm) (see also Table 1). These results indicate that more electron-withdrawing C13¹-substituents make more bathochromic shifts of redmost absorption bands of both cyclic and linear tetrapyrroles, **1b–3b** and **1c–3c**. Theoretically estimated values of the absorption maxima by molecular modeling calculation increased in the order of **2b** \rightarrow **1b** \rightarrow **3b** and **2c** \rightarrow **1c** \rightarrow **3c**, which supported experimental results (see Table S1). Redmost bands of **1c–3c** were broadened about 2.5 times more than those of **1b–3b** (see Table S1). The red-shifted and wider bands are characteristic of such linear tetrapyrroles prepared by photooxidation including similar compounds reported in [10,11] and **4c–6c** (vide infra). The bands of **2b** and **2c** at around 400 nm were more intense than the corresponding redmost bands, while

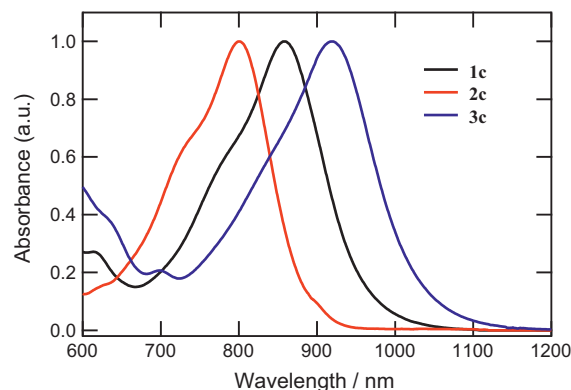


Fig. 3. Electronic absorption spectra (≥ 600 nm) of **1c–3c** in dichloromethane. Normalized at each intense peak.

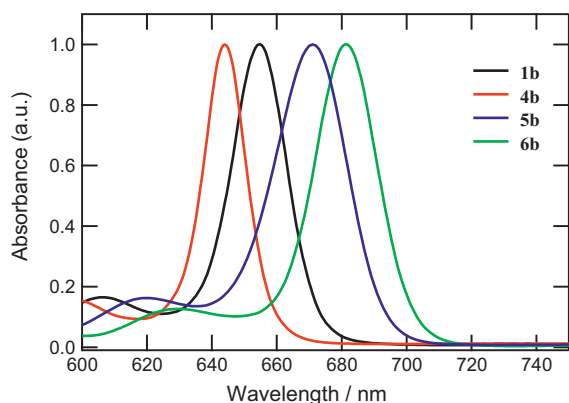


Fig. 4. Electronic absorption spectra (≥ 600 nm) of **1b** and **4b–6b** in dichloromethane. Normalized at each Qy peak.

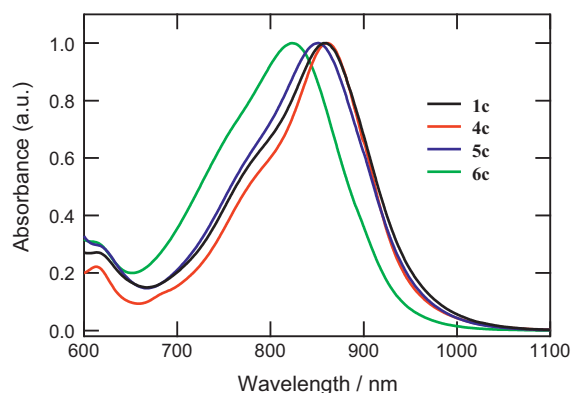


Fig. 5. Electronic absorption spectra (≥ 600 nm) of **1c** and **4c–6c** in dichloromethane. Normalized at each intense peak.

the two peak intensities of **1b/1c** and **3b/3c** were comparable (see Figs. S1 and S2). The decrease of the redmost peak intensity in **2b/2c** is ascribable to the lack of electron-withdrawing groups at the 13-position and is explained by the suppression of the molecular transition dipole moment along the y-axis [14].

The C3-substituent effect on electronic absorption spectra was investigated, in the same way as the C13-substituent effect mentioned above. Cyclic tetrapyrroles, **1b**, **4b**, **5b** and **6b** have vinyl, ethyl, *trans*-(methoxycarbonyl)ethenyl and formyl groups, respectively, at the 3-position. The electronegativity of the C3-functional group increased in the order of **4b** < **1b** < **5b** < **6b** [25]. Their Qy peaks are situated at 644, 655, 671 and 681 nm, respectively (Fig. 4). More electron-withdrawing C3-substituents induce larger red shifts of their Qy peaks as reported earlier [14]. On the contrary, redmost bands of the corresponding linear tetrapyrroles, **1c** and **4c–6c** were blue-shifted as an increase of the electronegativity of the C3-functional groups: absorption maxima/shoulders = 861/788 (**4c**) > 858/786 (**1c**) > 851/775 (**5c**) > 823/758 nm (**6c**) (Fig. 5). Molecular modeling calculation qualitatively predicted the reverse tendency for cyclic and linear tetrapyrroles possessing different C3-substituents (see Table S1). Electronic absorption spectra of linear tetrapyrroles **1c–6c** were fairly different from those of cyclic tetrapyrroles **1b–6b**, so the sensitivity of redmost bands by C3-substitution might be quite the reverse for cyclized **1b/4b–6b** and opened **1c/4c–6c**. In linear tetrapyrroles **1c–6c**, the C3- and C13-substituents are situated on the terminal A-ring and in-between C-ring, respectively, while the two substituents in cyclic tetrapyrroles **1b–6b** are similar on the pyrrole rings between two adjacent pyrrole rings. Such different situation for the C3-substituents of cyclic and linear tetrapyrroles would give the

reverse tendency and a similar situation for the C13-substituents would afford the same substitution effect. Moreover, the present oxidative ring-opening induced the direct formylation of the A-ring at the 1-position and no change around the substituents on the C-ring. The C1-formyl group in **1c/4c–6c** would make the C3-substituent effect to be the reverse as in **1b/4b–6b** possessing a substituted vinyl group at the 1-position.

None of the linear tetrapyrroles **1c–6c** examined here gave any apparent fluorescence emission bands in dichloromethane, while the corresponding cyclic tetrapyrroles **1b–6b** emitted fluorescence largely at the red side of Qy absorption maxima. Such an inefficient emission of **1c–6c** might be ascribable to more conformational flexibility of the present linear tetrapyrroles.

4. Conclusion

Using photooxidative cleavage of zinc chlorophyll derivatives **1b–6b**, zinc complexes of linear tetrapyrroles **1c–6c** having various substituents at the 3- and 13-positions were prepared systematically. Electron-withdrawing C3- and C13-substituents of the photocleaved products moved the redmost electronic absorption bands to shorter and longer wavelengths, respectively. The reverse substitution effects in linear tetrapyrroles **1c–6c** are different from the same effects of C3- and C13- substituents in the corresponding cyclic tetrapyrroles **1b–6b**. The six linear tetrapyrrole **1c–6c** examined here gave redmost maxima at 800–919 nm. Such linear tetrapyrroles should be useful for the development of near-infrared light absorbing pigments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2011.02.027.

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