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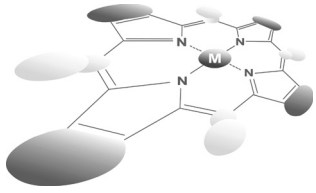

# Regioisomeric synthesis of chlorin-$e_6$ dimethyl esters and their optical properties

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**ABSTRACT:** Chlorin-$e_6$ dimethyl esters possessing a single carboxy group at the 13-, $15^1$-, or $17^2$-position were prepared by chemically modifying chlorophyll-*a*. These three synthetic regioisomers were fully characterized by their mass, NMR, and visible absorption spectra. Their molecular structures were unambiguously identified by the specific $^1$H–$^{13}$C correlation at the 13-, 15-, and/or 17-substituents in their respective HMBC spectra. Methyl esterification of 13/$15^1$-COOH and hydrolysis of 13/$15^1$-COOMe affected small shifts of the Qy absorption and fluorescence emission maxima in a diluted $CH_2Cl_2$ solution, while no substitution effect of $17^2$-COOH/Me was observed.

**KEYWORDS:** chlorophyll-*a* derivative, ester protection, fluorescence emission, Qy absorption, retro-Dieckmann condensation.



## INTRODUCTION

Chlorin-$e_6$ is one of the chlorophyll-*a* derivatives which is readily available through the retro-Dieckmann condensation opening of the *exo*-five-membered ring bearing a β-keto-ester moiety. Chlorin-$e_6$ and its derivatives are widely used as photosensitizers in medicinal [1] and material sciences [2]. Chlorin-$e_6$ has three carboxy groups in a molecule (Fig. 1) which are partially different in their reactivities, but their regioselectively chemical modification is difficult. Herein, we report on the preparation of regioselectively, doubly methyl-esterified chlorin-$e_6$ molecules **1a**–**1c** and reveal their optical properties in a solution, which are compared with those of trimethyl ester **2**.

One of the regioisomers, **1a**, has previously been prepared and its molecular structure was confirmed by chemical modification [3]. The synthetic route of another regioisomer **1c** was described [4], but its structure has not yet been identified. Moreover, **1b** has never been reported in any papers, to the best of our knowledge. In this paper, we mention fully synthetic procedures for obtaining regioisomers **1a**–**1c** and determine their molecular structures in their intact forms for the first time using a variety of $^1$H and $^{13}$C NMR techniques. With all the three regioisomers in hand, their visible absorption, circular dichroism (CD), and fluorescence emission spectra in a diluted dichloromethane solution were systematically compared and also their emission quantum yields and lifetimes were evaluated. The present regioisomers **1a**–**1c** possess a single carboxy group in a molecule and will be useful for the preparation of various regioisomerically pure chlorin-$e_6$ derivatives.

## RESULTS AND DISCUSSION

### Synthesis of chlorin-$e_6$ dimethyl ester regioisomers

Chlorophyll-*a* was extracted from commercially available cyanobacterial cells, *spirulina* powder, by methanol [5], readily demetalated to pheophytin-*a* by treatment of the extract with an aqueous diluted hydrochloric acid solution (step (i) in Scheme 1) [3, 5], and further hydrolyzed at the 17-propionate residue with trifluoroacetic acid to give pheophorbide-*a* (step (ii)) [3]. It is noted that the conditions of step (ii) promoted no more hydrolysis of the $13^2$-methoxycarbonyl group. During the

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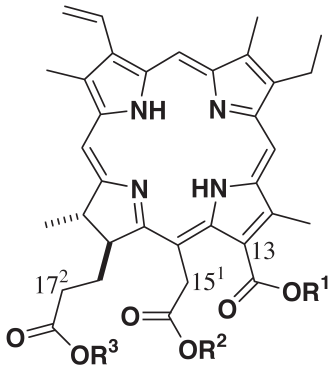



**1a**: $R^1$=H, $R^2$=$R^3$=Me
**1b**: $R^1$=$R^3$=Me, $R^2$=H
**1c**: $R^1$=$R^2$=Me, $R^3$=H

**Fig. 1.** Molecular structure of chlorin-$e_6$ ($R^1$=$R^2$=$R^3$=H) as well as its dimethyl esters **1a**–**1c** and trimethyl ester **2** ($R^1$=$R^2$=$R^3$=Me)

above procedures, the epimerization at the $13^2$-position occurred and the resulting pheophorbide-*a* contained about 20% of the ($13^2S$)-epimer, pheophorbide-*a*′ (prime form). The mixture of pheophorbides-*a*/*a*′ was dissolved in chloroform and treated with 0.5% (wt/v) potassium hydroxide in methanol at room temperature (step (iii)) [3, 4]. The basic conditions cleaved the $C13^1$–$C13^2$ bond of the *exo*-five-membered ring (E-ring) to afford chlorin-$e_6$ $13,15^1$-dimethyl ester (**1c**) as the methanol adduct (a retro-Diekmann condensation product) in a 61% isolated yield based on consumed pheophorbide-*a*/*a*′ after purification with flash column chromatography (FCC).

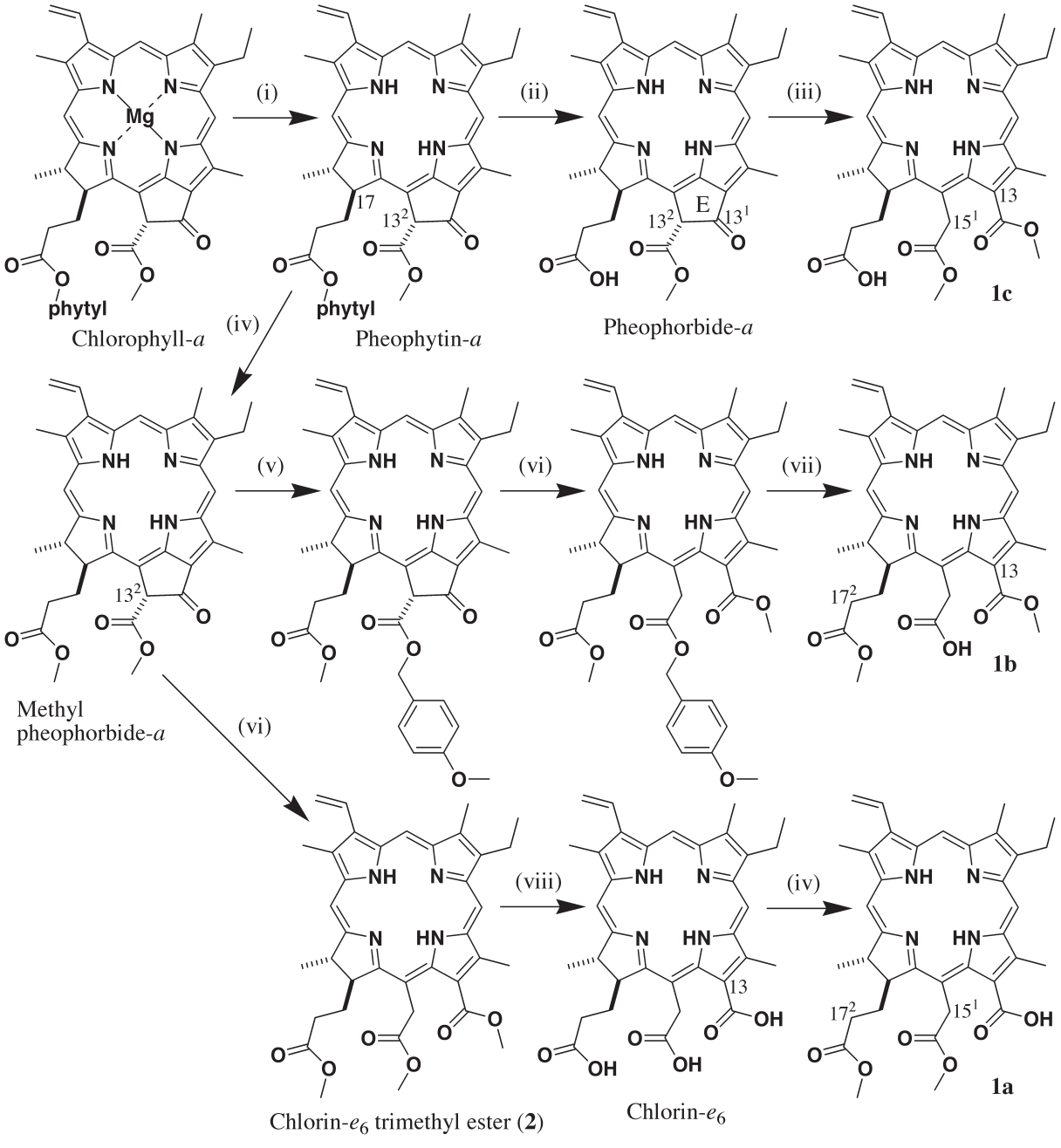
Methyl pheophorbide-*a* was obtained from chlorophyll-*a* *via* pheophytin-*a* (steps (i) and (iv)) according to the reported procedures [3, 5]. As mentioned above, the $13^2$-epimerization also occurred and about 10% of the prime form was observed in the product. Its methyl ester at the $13^2$-position was selectively transesterified with *p*-methoxybenzyl alcohol by action of 2-chloro-1-methylpyridinium iodide (Mukaiyama condensation reagent) and 4-(*N*,*N*-dimethylamino)pyridine (step (v), 46%) [6]. The product was subjected to similar basic conditions as the above step (iii) and the E-ring-cleaved product was produced in a 59% isolated yield based on consumed pheophorbide-*a* derivative (step (vi)). The prolonged reaction led to the transesterification of the desired product to trimethyl ester **2**, so it was important to frequently check the ring-opening reaction using TLC. The resulting *p*-methoxybenzyl ester was hydrolyzed by trifluoromethanesulfonic acid in trifluoroacetic acid (step (vii)) to afford chlorin-$e_6$ $13,17^2$-dimethyl ester (**1b**, 69%). It is noteworthy that no hydrolysis of methyl esters was found under acidic conditions [7].

Chlorin-$e_6$ $15^1,17^2$-dimethyl ester (**1a**) was obtained according to procedures reported previously [3, 8]. Its preparation is briefly described below. Methyl pheophorbide-*a* was converted to chlorin-$e_6$ trimethyl ester (**2**) by the same step (vi) as the aforementioned cleavage of the E ring [3]. The resulting three methyl esters were fully hydrolyzed with lithium iodide in refluxing ethyl acetate (step (viii)) [9]. Chlorin-$e_6$ bearing the three carboxy groups which is also available from some commercial suppliers was doubly and selectively esterified to give its $15^1,17^2$-dimethyl ester **1a** in a 41% isolated yield (step (iv)) [3, 8]. The regioselectivity can be attributed to less reactivity of the 13-carboxy group directly conjugated with chlorin π-system.

## Structural identification of chlorin-$e_6$ dimethyl ester regioisomers

All the three aforementioned products were purified with chromatography and showed the identical mass peak at *m*/z = 625 as $MH^+$ which is identical to that of chlorin-$e_6$ dimethyl ester ($C_{36}H_{41}N_4O_6$). The molecular structures of the dimethyl ester regioisomers were fully identified by the following NMR techniques in chloroform-*d* at room temperature. All single-set proton signals except two methyl esters ($COOCH_3$), one carboxylic acid (COOH), and two inner NH peaks were assigned by 1D and 2D $^1$H NMR spectra including COSY and ROESY (Figs S1–S3/S6–S8/S11–S13). All 36 carbon-13 peaks were recognized by 1D $^{13}$C NMR spectra including DEPT (Figs S4/S9/S14). Furthermore, two singlet proton signals of the methyl esters and almost all the $^{13}$C peaks were assignable by $^1$H–$^{13}$C HMQC and HMBC spectra (Figs S5/S10/S15).

The 15- and $17^1$-$CH_2$ proton signals were characterized at 5 to 6 ppm and 2 to 3 ppm, respectively. Three carbonyl carbon-13 signals were visible in a low field (169–178 ppm, see Table 1). In the HMBC spectra, one of the doublet proton peaks for the 15-$CH_2$ ($J$ = 19 Hz) was significantly correlated with one of the carbonyl $^{13}$C signals, which was assigned to the $C15^2$ peak (see Figs 2/S16/S17). A methylene proton peak at the $17^1$-position was clearly correlated with another carbonyl $^{13}$C signal in HMBC spectra and the $^{13}$C peak was unambiguously originated from the $C17^3$. The remaining carbonyl $^{13}$C signal was ascribed to the $C13^1$. In the HMBC spectrum of **1b** (Fig. 2), both the $C13^1$ and $C17^3$ peaks at 169.5 and 173.6 ppm were correlated with singlet proton peaks of methyl esters at 4.19 and 3.56 ppm, respectively, while the $C15^2$ peak at 176.7 ppm displayed no correlation with proton peaks at 3 to 5 ppm. The observation indicates that regioisomerically pure **1b** possesses 13-$COOCH_3$, $15^1$-COOH, and $17^2$-$COOCH_3$. Similarly, the HMBC spectral analyses indicate that **1a** and **1c** regioisomers have 13-COOH/$15^1$-$COOCH_3$/$17^2$-$COOCH_3$ and 13-$COOCH_3$/$15^1$-$COOCH_3$/$17^2$-COOH, respectively (Figs S16/S17). Table 1 shows the following two shifts for the $^{13}$C peaks of carbonyl groups. The esterification of COOH to $COOCH_3$ moves the carbonyl $^{13}$C signals to higher fields by 2.7–3.8 ppm and direct conjugation of the carbonyl




**Scheme 1.** Synthesis of chlorin-$e_6$ dimethyl esters **1a**–**1c** by chemical modification of naturally occurring chlorophyll-*a*: (i) aq. HCl, rt; (ii) aq. $CF_3COOH$, rt; (iii) 0.5% KOH/MeOH, rt; (iv) 5% $H_2SO_4$/MeOH, rt; (v) 4-$MeOC_6H_4CH_2OH$, 2-$ClC_5H_4NMe^+I^-$, 4-$Me_2NC_5H_5N$/PhMe, reflux; (vi) 0.5 M MeONa/MeOH, $CHCl_3$, rt; (vii) 10% $CF_3SO_3H/CF_3COOH$, 0 °C; (viii) LiI/AcOEt, reflux

groups with a chlorin π-system induces high-field shifts (≈4 ppm) of the peaks ($C15^2/C17^3 \rightarrow C13^1$).

## Optical properties of chlorin-$e_6$ dimethyl ester regioisomers

In a diluted dichloromethane solution, chlorin-$e_6$ dimethyl ester regioisomers **1a**–**1c** and trimethyl ester **2** gave almost the same visible absorption and CD spectra (Fig. 3). These spectra indicated that these esters were monomeric in the solution. A close inspection of the redmost Qy absorption band at around 665 nm (see Fig. 4a) showed small substitution effects. Hydrolysis of methyl ester at the 13-position as in **2** → **1a** moved the Qy maximum $\lambda_{abs}$ (Qy) to a longer wavelength by 1 nm, while that at the $15^1$-position as in **2** → **1b** did it to a shorter wavelength by 1 nm (Table 2). No shift of the Qy maximum was observed in the hydrolysis of methyl ester at the $17^2$-position as in **2** → **1c**. The carboxy and methoxycarbonyl groups at the 13-position are directly conjugated with chlorin π-system in a molecule and those at the $15^1$-position are homoconjugated with it

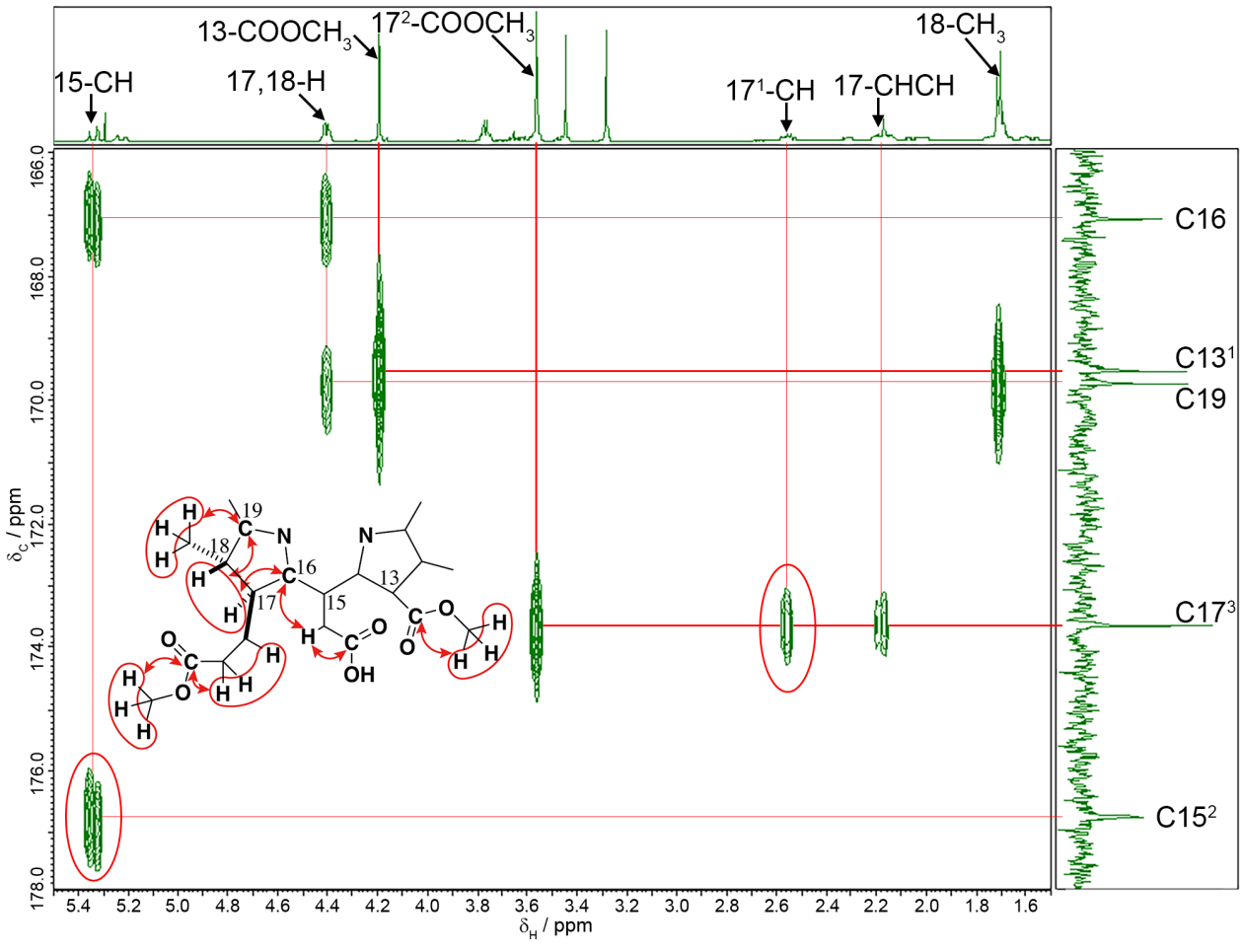



**Fig. 2.** Specific HMBC spectrum of **1b** in chloroform-*d* at room temperature and the $^{1}H$–$^{13}C$ correlation (red arrows) in the peripheral substituents at the 13-, 15-, 17-, and 18-positions (inset)

**Table 1.** Chemical shifts δs (ppm) for $^{1}H$ and $^{13}C$ signals of $COOCH_3$ and COOH of chlorin-$e_6$ dimethyl ester regioisomers **1a**–**1c** in chloroform-*d*

| Compound | COOR (R=$CH_3$ or H) | | | $COOCH_3$ | | | $COOCH_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | 13- | $15^1$- | $17^2$- | 13- | $15^1$- | $17^2$- | 13- | $15^1$- | $17^2$- |
| **1a** (13-COOH) | 173.2 | 174.0 | 173.5 | — | 52.5 | 51.6 | — | 3.80 | 3.58 |
| **1b** ($15^1$-COOH) | 169.5 | 176.7 | 173.6 | 53.2 | — | 51.7 | 4.19 | — | 3.56 |
| **1c** ($17^2$-COOH) | 169.5 | 173.2 | 177.3 | 53.0 | 52.1 | — | 4.22 | 3.73 | — |

through one $sp^3$-carbon atom, so they slightly affected the Qy absorption bands. These functional groups at the $17^2$-position are connected with chlorin π-core *via* four single C–C bonds and far from the π-system, resulting in no effect on the visible absorption bands. These observations are consistent with the reported data for chlorophyll derivatives with various esterifying groups at the 17-propionate residue [10]. It is noted that no apparent effect was found in their Soret bands at 403 nm.

When the diluted dichloromethane solutions (*ca.* 1 μM) of **1a**–**1c** and **2** were excited at the Soret maxima, an intense emission band was observed as the mirror image of the Qy band with approximately 150 $cm^{-1}$ of Stokes shift Δ (Fig. 4b and Table 2). A small red shift (1 nm) of fluorescence emission maximum $\lambda_{em}$ of **1a** over **1b/1c/2** is ascribable to the aforementioned bathochromic shift of the Qy maximum $\lambda_{abs}$ (Qy) of the former bearing the 13-COOH over the latter possessing the 13-$COOCH_3$. Their fluorescence emission quantum yields $\Phi_{em}$ are almost the same, 16–17% (Table 2). All the fluorescence emissions were single-exponentially decayed to give virtually identical lifetimes $\tau_{em}$ (about 4.5 ns). Apparent substitution effects on $\Phi_{em}$ and $\tau_{em}$ are invisible for **1a**–**1c** and **2**.

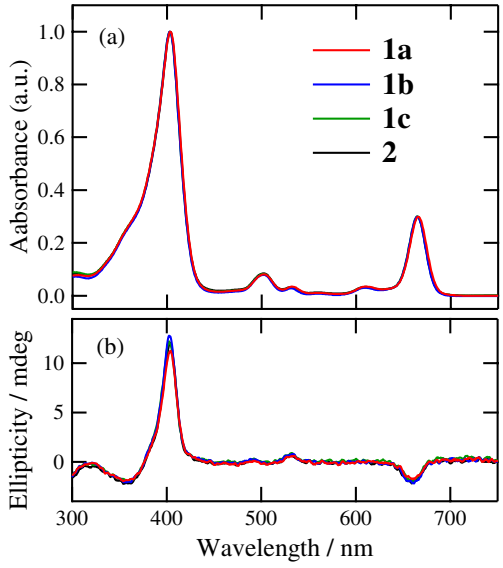



**Fig. 3.** Visible absorption (a) and CD spectra (b) of chlorin-$e_6$ dimethyl ester regioisomers **1a**–**1c** and trimethyl ester **2** in dichloromethane (*ca.* 10 μM) at room temperature. All the absorption spectra were normalized at their intense peaks at the Soret region

## EXPERIMENTAL

### General

All melting points were measured with a Yanagimoto micro melting apparatus and were uncorrected. Visible absorption and CD spectra were measured with Hitachi U-3500 and Jasco J-720W spectrophotometers, respectively. The fluorescence emission spectra and quantum yields excited at Soret maxima were obtained by a Hamamatsu Photonics C9920-03G spectrometer. Fluorescence emission lifetimes excited at 403 nm were determined with a Hamamatsu Photonics C7990S system. $^1$H and $^{13}$C NMR spectra were recorded on a JEOL ECA-600 (600 MHz) spectrometer; tetramethylsilane (0.00 ppm) was used as an internal reference. Proton and carbon-13 peaks were assigned using $^1$H–$^1$H COSY/ROESY, $^{13}$C DEPT, and $^1$H–$^{13}$C HMQC/HMBC techniques. Standard mass data were obtained using laser desorption/ionization (LDI) by a Shimadzu AXIMA-CFR plus spectrometer. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF II spectrometer: atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) and positive mode in a methanol solution. FCC and TLC were performed with silica gel (Wakogel C-300 and Merck Kieselgel 60 $F_{254}$). RP-HPLC was performed by a Shimadzu LC-10AD$_{VP}$ pump, SPD-M10A$_{VP}$ diode-array detector, and SCL-10A$_{VP}$ system controller using a packed ODS column (Nacalai Tesque Cosmosil 5$C_{18}$-AR-II, 10 mmϕ × 250 mm).

Chlorin-$e_6$ [9], chlorin-$e_6$ trimethyl ester (**2**) [3], methyl pheophorbide-*a*/*a*′ [3, 5], and pheophorbide-*a*/*a*′ [3] were prepared according to reported procedures. All the reaction reagents and solvents were obtained from commercial suppliers and utilized as supplied. Dry toluene was obtained by reflux with calcium chloride and distillation. Distilled water was prepared by a Yamato AutoStill WG250 system. All the reactions were performed in the dark. Dichloromethane for optical spectroscopy was purchased from Nacalai Tesque as a reagent prepared specially for spectroscopy and used without further purification.

Before measurements of optical properties, the sample was purified by RP-HPLC with MeOH : $H_2O$ : $CF_3COOH$ = 75 : 25 : 0.1 (1.0 ml/min). The desired HPLC elution was diluted with dichloromethane, washed

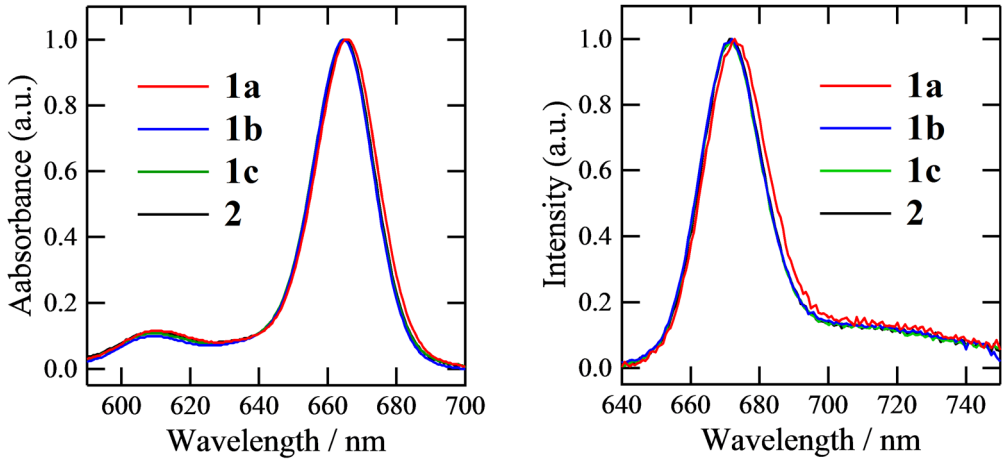



**Fig. 4.** Visible absorption (a), *ca.* 10 μM and fluorescence emission spectra (b), *ca.* 1 μM of chlorin-$e_6$ dimethyl ester regioisomers **1a**–**1c** and trimethyl ester **2** in aerated dichloromethane at room temperature. All the spectra were normalized at their intense peaks at the Qy region and the emission spectra were measured by excitation at the Soret maxima

**Table 2.** Optical properties of chlorin-$e_6$ dimethyl ester regioisomers **1a**–**1c** and trimethyl ester **2** in aerated dichloromethane at room temperature[a]

| Compound | $\lambda_{abs}$/nm | | $\lambda_{em}$/nm | $\Delta$/cm$^{-1}$ | $\Phi_{em}$/% | $\tau_{em}$/ns |
|---|---|---|---|---|---|---|
| | Soret | Qy | | | | |
| **1a** (13-COOH) | 403 (2090) | 666 (490) | 672 (480) | 140 | 16 | 4.3 |
| **1b** (15$^1$-COOH) | 403 (2040) | 664 (470) | 671 (490) | 160 | 16 | 4.6 |
| **1c** (17$^2$-COOH) | 403 (2100) | 665 (480) | 671 (470) | 150 | 17 | 4.6 |
| **2** | 403 (2050) | 665 (450) | 671 (480) | 140 | 17 | 4.5 |

[a] $\lambda_{abs}$, intense absorption maximum; $\lambda_{em}$, emission maximum (excited at Soret maxima); $\Delta$, Stokes shift = $[1/\lambda_{abs}\,(Qy) - 1/\lambda_{em}] \times 10^7$; $\Phi_{em}$, emission quantum yield (excited at Soret maxima); $\tau_{em}$, emission lifetime (excited at 403 nm); the values in parentheses indicate full widths at half maxima of bands (cm$^{-1}$).



with distilled water, and dried over sodium sulfate. After evaporation, the residue was dissolved in dichloromethane and the solution was optically analyzed.

### Synthesis of chlorin-$e_6$ 15$^1$,17$^2$-dimethyl ester (1a)

Chlorin-$e_6$ (35.4 mg, 59.3 μmol) was dissolved in a 5% (v/v) methanol solution of sulfuric acid and stirred at room temperature under argon for 14 h. After disappearance of the starting material checked by TLC ($CH_2Cl_2$ : MeOH = 10 : 1), the reaction mixture was poured into ice-chilled water and extracted with dichloromethane several times. The combined organic phases were washed with an aqueous 4% (wt/v) sodium hydrogen carbonate and brine, dried over sodium sulfate, and filtered. All the solvents were evaporated and the residue was purified with FCC ($CH_2Cl_2$ : MeOH = 10 : 1) and recrystallization from dichloromethane and hexane to give **1a** (15.2 mg, 24.3 μmol) in a 41% yield (95% in [3]): bluish black solid; mp = 155–156 °C; VIS ($CH_2Cl_2$) $\lambda_{max}$ = 666 (relative intensity, 0.30), 610 (0.03), 532 (0.03), 502 (0.08), 403 nm (1.00); $^1$H NMR (600 MHz, $CDCl_3$) δ = 9.60 (1H, s, 10-H), 9.50 (1H, s, 5-H), 8.71 (1H, s, 20-H), 8.03 (1H, dd, *J* = 18, 12 Hz, 3$^1$-H), 6.33 (1H, dd, *J* = 18, 1 Hz, 3$^2$-H *trans* to 3-C–H), 6.12 (1H, dd, *J* = 12, 1 Hz, 3$^2$-H *cis* to 3-C–H), 5.54, 5.26 (each 1H, d, *J* = 19 Hz, 15-$CH_2$), 4.41 (1H, q, *J* = 7 Hz, 18-H), 4.38 (1H, d, *J* = 10 Hz, 17-H), 3.80 (3H, s, 15$^1$-$COOCH_3$), 3.69 (2H, q, *J* = 8 Hz, 8-$CH_2$), 3.58 (6H, s, 12-$CH_3$, 17$^2$-$COOCH_3$), 3.45 (3H, s, 2-$CH_3$), 3.25 (3H, s, 7-$CH_3$), 2.57–2.48 (1H, m, 17$^1$-CH), 2.20–2.10 (2H, m, 17-CHCH), 1.76 (3H, d, *J* = 7 Hz, 18-$CH_3$), 1.70 (1H, br, 17-CH), 1.65 (3H, t, *J* = 8 Hz, 8$^1$-$CH_3$), –1.33 (1H, s, NH) [the 13-COOH and one of the two NH signals could be invisible]; $^{13}$C NMR ($CDCl_3$, 150 MHz) δ = 174.0 (C15$^2$), 173.5 (C17$^3$), 173.2 (C13$^1$), 169.7 (C19), 167.2 (C16), 154.9 (C6), 148.8 (C9), 145.0 (C8), 139.6 (C1), 137.0, 129.3 (C11, 12), 136.1 (C14), 135.8 (C7), 135.5 (C4), 134.8 (C3), 130.6 (C2), 129.4 (C3$^1$), 123.1 (C13), 121.7 (C3$^2$), 102.4 (C10), 102.2 (C15), 98.6 (C5), 93.6 (C20), 52.8 (C17), 52.5 (C15$^4$), 51.6 (C17$^5$), 49.5 (C18), 39.2 (C15$^1$), 31.1 (C17$^2$), 29.3 (C17$^1$), 22.7 (C18$^1$), 19.6 (C8$^1$), 17.6 (C8$^2$), 12.6 (C12$^1$), 12.1 (C2$^1$), 11.3 (C7$^1$); HRMS (ESI) found: *m/z* 625.3021, calcd for $C_{36}H_{41}N_4O_6$: $MH^+$, 625.3021.

### Synthesis of chlorin-$e_6$ 13,17$^2$-dimethyl ester (1b)

Methyl pheophorbide-*a*/*a*′ (7/1, 101.6 mg, 167 μmol) was dissolved in dry toluene (10 ml), to which were added 2-chloro-1-methylpyridinium iodide (132.2 mg, 517 μmol, 3.1 eq.), 4-(*N*,*N*-dimethylamino)pyridine (136.4 mg, 1.116 mmol, 6.7 eq.), and 4-methoxybenzyl alcohol (37.7 mg, 273 μmol, 1.6 eq.). The mixture was refluxed under nitrogen for 3 h and cooled down to room temperature: the reaction was monitored by TLC ($CH_2Cl_2$ : $Et_2O$ = 100 : 3) and mass analysis (*m/z* = 712 for $M^+$ of the product). After evaporation of all the solvent, the residue was purified with FCC ($CH_2Cl_2$ : $Et_2O$ = 100 : 3) to give methyl (13$^2$*R*/*S*)-(4-methoxybenzyloxycarbonyl) pyropheophorbide-*a* (10/1, 54.4 mg, 76.3 μmol, 46%): bluish black solid; mp = 178–179 °C; UV-vis ($CH_2Cl_2$) $\lambda_{max}$ = 668 (relative intensity, 0.43), 611 (0.08), 539 (0.10), 508 (0.11), 414 nm (1.00); $^1$H NMR (600 MHz, $CDCl_3$) δ(10/1) = 9.37/9.33 (1H, s, 10-H), 9.18/9.15 (1H, s, 5-H), 8.49/8.47 (1H, s, 20-H), 7.85 (1H, dd, *J* = 18, 12 Hz, 3$^1$-H), 7.30 (2H, d, *J* = 8 Hz, 2,6-H of 13$^2$-COOCAr), 6.78 (2H, d, *J* = 8 Hz, 3,5-H of 13$^2$-COOCAr), 6.18 (1H, dd, *J* = 18, 1 Hz, 3$^2$-H *trans* to 3-C–H), 6.09 (1H, dd, *J* = 12, 1 Hz 3$^2$-H *cis* to 3-C–H), 5.37, 5.28/5.32, 5.23 (each 1H, d, *J* = 12 Hz, 13$^2$-$COOCH_2$), 4.35/4.45 (1H, dq, *J* = 2, 7 Hz, 18-H), 3.93/3.90 (1H, dt, *J* = 9, 2 Hz, 17-H), 3.74/3.72 (3H, s, 4-$OCH_3$ of 13$^2$-COOCAr), 3.65 (3H, s, 12-$CH_3$), 3.55 (3H, s, 17$^2$-$COOCH_3$), 3.51 (2H, q, *J* = 8 Hz, 8-$CH_2$), 3.33 (3H, s, 2-$CH_3$), 3.07/3.05 (3H, s, 7-$CH_3$), 2.57–2.52, 2.29–2.22 (each 1H, m, 17-$CH_2$), 2.36–2.31, 2.17–2.12 (each 1H, m, 17$^1$-$CH_2$), 1.61 (3H, t, *J* = 8 Hz, 8$^1$-$CH_3$), 1.61 (3H, d, *J* = 7 Hz, 18-$CH_3$), 0.39, –1.78/0.53, –1.56 (each 1H, s, NH × 2); HRMS (APCI) found: *m/z* 713.3330, calcd for $C_{43}H_{45}N_4O_6$: $MH^+$, 713.3334.

The above 4-methoxybenzyl ester (48.4 mg, 67.9 μmol) was dissolved in chloroform (5 ml), to which was added methanol (10 ml) and successively a 0.5 M methanol solution (5 ml) of sodium methoxide was

dropped. The mixture was stirred at room temperature under nitrogen for 1 h. After detection of a more movable spot on TLC ($CH_2Cl_2$ : $Et_2O$ = 100 : 3) and a new mass peak at *m/z* = 744, the reaction mixture was quenched with water, diluted with chloroform, washed with water, dried over sodium sulfate, and evaporated. The residue was purified with FCC ($CH_2Cl_2$ : $Et_2O$ = 100: 3) to give chlorin-$e_6$ 13,17$^2$-dimethyl-15$^1$-(4-methoxybenzyl) ester (the first fraction, 28.3 mg, 37.9 μmol, 56%) with recovery of the starting material (the second fraction, 2.8 mg, 3.9 μmol, 6%): bluish black solid; mp = 109–110 °C; UV-vis ($CH_2Cl_2$) $\lambda_{max}$ = 660 (relative intensity, 0.30), 610 (0.04), 531 (0.04), 502 (0.09), 403 nm (1.00); $^1$H NMR (600 MHz, $CDCl_3$) δ = 9.70 (1H, s, 10-H), 9.55 (1H, s, 5-H), 8.73 (1H, s, 20-H), 8.05 (1H, dd, *J* = 18, 12 Hz, 3$^1$-H), 7.23 (2H, d, *J* = 9 Hz, 2,6-H of 15$^1$-COOCAr), 6.76 (2H, d, *J* = 9 Hz, 3,5-H of 15$^1$-COOCAr), 6.18 (1H, d, *J* = 18 Hz, 3$^2$-H *trans* to 3-C–H), 6.13 (1H, d, *J* = 12 Hz 3$^2$-H *cis* to 3-C–H), 5.22, 5.14 (each 1H, d, *J* = 12 Hz, 15$^1$-$CH_2$), 4.39 (1H, q, *J* = 7 Hz, 18-H), 4.35 (1H, d, *J* = 10 Hz, 17-H), 4.16 (3H, s, 13-$COOCH_3$), 3.78 (2H, q, *J* = 7 Hz, 8-$CH_2$), 3.74 (3H, s, 4-$OCH_3$ of 15$^1$-COOCAr), 3.60 (3H, s, 17$^2$-$COOCH_3$), 3.58 (3H, s, 12-$CH_3$), 3.46 (3H, s, 2-$CH_3$), 3.29 (3H, s, 7-$CH_3$), 2.57–2.50 (1H, m, 17$^1$-CH), 2.20–2.13 (2H, m, 17-CHCH), 1.72 (3H, t, *J* = 7 Hz, 18-$CH_3$), 1.71 (1H, br, 17-CH), 1.65 (3H, d, *J* = 7 Hz, 8$^1$-$CH_3$), 0.10, –1.47 (each 1H, s, NH × 2) [the corresponding mono-benzyl ester showed a similar $^1$H NMR spectrum, see [11]]; HRMS (APCI) found: *m/z* 745.3595, calcd for $C_{44}H_{49}N_4O_7$: $MH^+$, 745.3595.

Chlorin-$e_6$ 13,17$^2$-dimethyl-15$^1$-(4-methoxybenzyl) ester (8.5 mg, 11.4 μmol) was dissolved into 10% (v/v) trifluoromethanesulfonic acid in trifluoroacetic acid (4 ml) and stirred at 0 °C under nitrogen for 1 h. After TLC (full disappearance of 4-methoxybenzyl ester at $R_f$ = 0.88 and appearance of carboxylic acid at $R_f$ = 0.5 in $CH_2Cl_2$ : MeOH = 10 : 1) and mass analyses (detection of peak at *m/z* = 624), the reaction mixture was diluted with dichloromethane, washed with water three times, dried over sodium sulfate, and filtered. The solvents were evaporated and the residue was purified with FCC ($CH_2Cl_2$ : MeOH = 10 : 1) and recrystallization from dichloromethane and hexane to give **1b** (4.9 mg, 7.8 μmol, 69%): bluish black solid; mp = 162–164 °C; UV-vis ($CH_2Cl_2$) $\lambda_{max}$ = 664 (relative intensity, 0.30), 610 (0.03), 531 (0.03), 502 (0.08), 403 nm (1.00); $^1$H NMR (600 MHz, $CDCl_3$) δ = 9.68 (1H, s, 10-H), 9.54 (1H, s, 5-H), 8.71 (1H, s, 20-H), 8.04 (1H, dd, *J* = 18, 12 Hz, 3$^1$-H), 6.34 (1H, dd, *J* = 18, 2 Hz, 3$^2$-H *trans* to 3-C–H), 6.13 (1H, dd, *J* = 12, 1 Hz, 3$^2$-H *cis* to 3-C–H), 5.34, 5.23 (each 1H, d, *J* = 19 Hz, 15-$CH_2$), 4.41 (1H, q, *J* = 7 Hz, 18-H), 4.40 (1H, d, *J* = 10 Hz, 17-H), 4.19 (3H, s, 13-$COOCH_3$), 3.77 (2H, q, *J* = 7 Hz, 8-$CH_2$), 3.564 (3H, s, 17$^2$-$COOCH_3$), 3.559 (3H, s, 12-$CH_3$), 3.45 (3H, s, 2-$CH_3$), 3.28 (3H, s, 7-$CH_3$), 2.61–2.53 (1H, m, 17$^1$-CH), 2.23–2.13 (2H, m, 17-CHCH), 1.71 (3H, d, *J* = 7 Hz, 18-$CH_3$), 1.70 (3H, t, *J* = 7 Hz, 8$^1$-$CH_3$), 1.69 (1H, br, 17-CH), –1.45 (1H, s, NH) [the 15-COOH and one of the two NH signals could be invisible]; $^{13}$C NMR ($CDCl_3$, 150 MHz) δ = 176.7 (C15$^2$), 173.6 (C17$^3$), 169.7 (C19), 169.5 (C13$^1$), 167.1 (C16), 154.9 (C6), 148.9 (C9), 145.1 (C8), 139.6, 130.6 (C1, 2), 136.6, 129.3 (C11, 12), 136.0 (C7), 135.4 (C4), 135.3 (C14), 134.8 (C3), 129.3 (C3$^1$), 123.1 (C13), 121.9 (C3$^2$), 102.3 (C10), 101.5 (C15), 98.7 (C5), 93.6 (C20), 53.2 (C13$^3$), 52.8 (C17), 51.7 (C17$^5$), 49.4 (C18), 38.4 (C15$^1$), 31.1 (C17$^2$), 29.4 (C17$^1$), 22.8 (C18$^1$), 19.7 (C8$^1$), 17.7 (C8$^2$), 12.4 (C12$^1$), 12.1 (C2$^1$), 11.3 (C7$^1$); HRMS (APCI) found: *m/z* 625.3026, calcd for $C_{36}H_{41}N_4O_6$: $MH^+$, 625.3021.

### Synthesis of chlorin-$e_6$ 13,15$^1$-dimethyl ester (1c)

Pheophorbide-*a*/*a*′ (4/1, 78.5 mg, 131.6 μmol) was dissolved in chloroform (5 ml), to which was added a 0.5% (wt/v) methanol solution (20 ml) of potassium hydroxide. The mixture was stirred at room temperature under nitrogen for 10 h. After detection of a more movable spot on TLC ($CH_2Cl_2$ : MeOH = 10 : 1), the reaction mixture was quenched with an aqueous 5% (wt/v) citric acid solution and extracted with chloroform several times. The combined organic phases were dried over sodium sulfate, filtered, and evaporated. The residue was purified with FCC ($CH_2Cl_2$ : MeOH = 10 : 1) and recrystallization from dichloromethane and hexane to give **1c** (the first fraction, 45.1 mg, 72.2 μmol, 55%) with recovery of the starting material (the second fraction, 8.2 mg, 13.7 μmol, 10%): bluish black solid; mp = 220–221 °C; UV-vis ($CH_2Cl_2$) $\lambda_{max}$ = 665 (relative intensity, 0.30), 609 (0.03), 531 (0.03), 502 (0.08), 403 nm (1.00); $^1$H NMR (600 MHz, $CDCl_3$) δ = 9.68 (1H, s, 10-H), 9.55 (1H, s, 5-H), 8.73 (1H, s, 20-H), 8.05 (1H, dd, *J* = 18, 12 Hz, 3$^1$-H), 6.34 (1H, dd, *J* = 18, 1 Hz, 3$^2$-H *trans* to 3-C–H), 6.13 (1H, dd, *J* = 12, 1 Hz, 3$^2$-H *cis* to 3-C–H), 5.31, 5.26 (each 1H, d, *J* = 19 Hz, 15-$CH_2$), 4.43 (1H, q, *J* = 7 Hz, 18-H), 4.42 (1H, d, *J* = 10 Hz, 17-H), 4.22 (3H, s, 13-$COOCH_3$), 3.77 (2H, q, *J* = 8 Hz, 8-$CH_2$), 3.73 (3H, s, 15$^1$-$COOCH_3$), 3.56 (3H, s, 12-$CH_3$), 3.45 (3H, s, 2-$CH_3$), 3.28 (3H, s, 7-$CH_3$), 2.64–2.58 (1H, m, 17$^1$-CH), 2.25–2.16 (2H, m, 17-CHCH), 1.74 (3H, d, *J* = 7 Hz, 18-$CH_3$), 1.73 (1H, br, 17-CH), 1.70 (3H, t, *J* = 8 Hz, 8$^1$-$CH_3$), –1.48 (1H, s, NH) [the 17$^2$-COOH and one of the two NH signals could be invisible]; $^{13}$C NMR ($CDCl_3$, 150 MHz) δ = 177.3 (C17$^3$), 173.2 (C15$^2$), 169.5 (C13$^1$), 169.4 (C19), 166.7 (C16), 154.8 (C6), 148.9 (C9), 145.0 (C8), 139.5 (C1), 136.4, 129.3 (C11, 12), 136.0 (C7), 135.4, 135.4 (C4, 14), 134.8 (C3), 130.6 (C2), 129.4 (C3$^1$), 123.4 (C13), 121.8 (C3$^2$), 102.2, 102.2 (C10, 15), 98.7 (C5), 93.6 (C20), 53.0 (C13$^3$), 52.9 (C17), 52.1 (C15$^4$), 49.4 (C18), 38.6 (C15$^1$), 30.6 (C17$^2$), 29.2 (C17$^1$), 22.9 (C18$^1$), 19.7 (C8$^1$), 17.7 (C8$^2$), 12.4 (C12$^1$), 12.1 (C2$^1$), 11.3 (C7$^1$); HRMS (ESI) found: *m/z* 625.3021, calcd for $C_{36}H_{41}N_4O_6$: $MH^+$, 625.3021.

## CONCLUSION

Three regioisomerically pure chlorin-$e_6$ dimethyl esters **1a**–**1c** were prepared by chemical modification of naturally occurring chlorophyll-*a*. 13-Carboxylated chlorin-$e_6$ $15^1$,$17^2$-dimethyl ester (**1a**) was obtained by regioselective methylation at the $15^1$- and $17^1$-carboxy groups of chlorin-$e_6$ over its 13-COOH. $15^1$-Carboxylated chlorin-$e_6$ 13,$17^2$-dimethyl ester (**1b**) was prepared through protection of the $15^1$-carboxy group with *p*-methoxybenzyl ester. $17^2$-Carboxylated chlorin-$e_6$ 13,$15^1$-dimethyl ester (**1c**) was synthesized by the E-ring cleavage of pheophorbide-*a* under basic conditions. The molecular structures of the three regioisomers were fully identified by 1D and 2D NMR techniques including $^1$H–$^{13}$C HMBC. The synthetic regioisomers showed almost the same visible absorption, CD, and fluorescence emission spectra as well as emission quantum yields and lifetimes in a diluted dichloromethane solution. Small substitution effects were observed in Qy absorption and fluorescence emission bands by methyl-esterification at the 13- and $15^1$-carboxy groups, but not at the $17^2$-COOH.

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### Supporting information

Figures S1–S17 are given in the supplementary material. This material is available free of charge *via* the Internet at http://www.worldscinet.com/jpp/jpp.shtml.

## REFERENCES

1. (a) Celli JP, Spring BQ, Rizvi I, Evans CL, Samkoe KS, Verma S, Pogue BW and Hasan T. *Chem. Rev.* 2010; **110**: 2795–2838. (b) Huang X, Tian X-J, Yang W, Ehrenberg B and Chen J-Y. *Phys. Chem. Chem. Phys.* 2013; **15**: 15727–15733. (c) Bastien E, Schneider R, Hackbarth S, Dumas D, Jasniewski J, Röder B, Bezdetnaya L and Lassalle, H-P. *Photochem. Photobiol. Sci.* 2015; **14**: 2203–2212. (d) Liu G, Qin H, Amano T, Murakami T and Komatsu N. *ACS Appl. Mater. Interfaces* 2015; **7**: 23402–23406. (e) Jinadasa RGW, Zhou Z, Vicente MGH and Smith KM. *Org. Biomol. Chem.* 2016; **14**: 1049–1064. (f) Isaac-Lam MF and Hammonds DM. *Pharmaceuticals* 2017; **10**: 41. (g) Chen H, Humble SW, Jinadasa RGW, Zhou Z, Nguyen AL, Vicente MGH and Smith KM. *J. Porphyrins Phthalocyanines* 2017; **21**: 354–363. (h) Yaghini E, Dondi R, Tewari KM, Loizidou M, Eggleston IM and MacRobert AJ. *Sci. Rep.* 2017; **7**: 6059. (i) Cao L, Guo X, Wang L, Wang S, Li Y and Zhao W. *New J. Chem.* 2017; **41**: 14279–14287. (j) Otvagin VF, Nyuchev AV, Kuzmina NS, Grishin ID, Gavryushin AE, Romanenko YV, Koifman OI, Belykh DV, Peskova NN, Shilyagina NY, Balalaeva IV and Fedorov AY. *Eur. J. Med. Chem.* 2018; **144**: 740–750. (k) Srdanović S, Gao Y-H, Chen D-Y, Yan Y-J, Margetić D and Chen Z-L. *Bioorg. Med. Chem. Lett.* 2018; **28**: 1785–1791. (l) Phuong PTT, Lee S, Lee C, Seo B, Park S, Oh KT, Lee ES, Choi H-G, Shin BS and Youn YS. *Colloids Surf. B: Biointerfaces* 2018; **171**: 123–133.
2. (a) Wang X-F and Tamiaki H. *Energy Environ. Sci.* 2010; **3**: 94–106. (b) Wang X-F and Kitao O. *Molecules* 2012; **17**: 4484–4497. (c) Wang X-F, Tamiaki H, Kitao O, Ikeuchi T and Sasaki S. *J. Power Sources* 2013; **242**: 860–864.
3. Hargus JA, Fronczek FR, Vicente MGH and Smith KM. *Photochem. Photobiol.* 2007; **83**: 1006–1015.
4. Zhang L, Yao J-Z, Zheng H, Wang X-Y and Zhang W-N. *Chin. J. Pharm.* 2003; **34**: 371–372.
5. Tamiaki H, Takeuchi S, Tsudzuki S, Miyatake T and Tanikaga R. *Tetrahedron* 1998; **54**: 6699–6718.
6. (a) Shinoda S and Osuka A. *Tetrahedron Lett.* 1996; **37**: 4945–4948. (b) Furukawa H, Oba T, Tamiaki H and Watanabe T. *Bull. Chem. Soc. Jpn.* 2000; **73**: 1341–1351.
7. Tamiaki H, Hagio N, Tsuzuki S, Cui Y, Zouta T, Wang X-F and Kinoshita Y. *Tetrahedron* 2018; **74**: 4078–4085.
8. Guo X, Wang L, Wang S, Li Y, Zhang F, Song B and Zhao W. *Bioorg. Med. Chem. Lett.* 2015; **25**: 4078–4081.
9. Jinadasa RGW, Hu X, Vicente MGH and Smith KM. *J. Med. Chem.* 2011; **54**: 7464–7476.
10. (a) Tamiaki H, Shibata R and Mizoguchi T. *Photochem. Photobiol.* 2007; **83**: 152–162. (b) Shoji S, Hashishin T, Tamiaki H. *Chem. Eur. J.* 2012; **18**: 13331–13341. (c) Mizoguchi T, Isaji M, Yamano N, Harada J, Fujii R and Tamiaki H. *Biochemistry* 2017; **56**: 3682–3688.
11. Chen H, Jinadasa RGW, Jiao L, Fronczek FR, Nguyen AL and Smith KM. *Eur. J. Org. Chem.* 2015: 3661–3665.