



Stereoselective reduction, methylation, and phenylation of the 13-carbonyl group in chlorophyll derivatives

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ABSTRACT

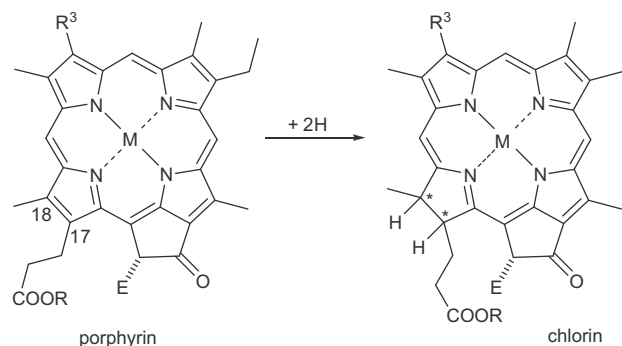
Regioselective reduction of the 13-carbonyl group on the five-membered exo-ring of methyl pyropheophorbide-*a*, one of the chlorophyll-*a* derivatives, with sodium borohydride gave an epimeric mixture of (13¹*R/S*)-hydroxy-chlorins. The stereoselectivity was controlled by the steric effect of the (17*S*)-methoxycarbonyl group to afford the (13¹*S*)-rich secondary alcohol (25% de). The use of sterically large lithium tri(*sec*-butyl)borohydride as the reductant enhanced the stereoselectivity to 55% de. The regio- and stereoselective methylation and phenylation of the 13-C=O of pyropheophorbide-*a* were observed using methyl and phenyl lithium, respectively. The major diastereomer of the tertiary alcohols obtained had the same configuration at the 13¹-stereogenic center as in the reduced product. All of the anion species (H[−], CH₃[−], and C₆H₅[−]) favorably attacked the 13¹-carbon atom from the reverse side of the 17-propionate residue, that is, the less sterically crowded face of the 13-C=O plane.

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1. Introduction

Asymmetric hydrogenation at the C=C double bond is observed in the biosynthesis of (bacterio)chlorophylls [(B)Chls]. For example, the C17=C18 bond in protochlorophyllide-*a* is enzymatically reduced to the C17H(*S*)-C18H(*S*) array in chlorophyllide-*a* (Scheme 1a).¹ It is difficult to mimic such a stereoselective hydrogenation in solution, and the regioselective hydrogenation at the C17=C18 bond was only achieved by photoreduction of the zinc protochlorophyll-*a* derivatives without any chiral control (Scheme 1b).²

In contrast, the stereoselective reduction of the 3-acetyl group in (B)Chl derivatives was reported to take place in solution using a chiral catalyst (Scheme 2a).³ The synthetic procedures are effective for the preparation of BChl-*d* and its analogues possessing a chiral 1-hydroxyethyl group at the 3-position. Reduction of the 3-COCH₃ in a Chl-*a* derivative bearing (17*S*,18*S*)-stereochemistry with an achiral reagent gave a 1:1 3¹-epimeric mixture of the secondary alcohol (Scheme 2b).⁴ No chiral induction was observed, partially because the reactive site (C3¹) was distant from the stereogenic centers (C17 and C18) in the molecule: a 1,7/8-induction through six/seven bonds between the C3¹ and C18/17 positions. A similar reduction of a BChl-*a* derivative bearing (7*R*,8*R*)-stereochemistry also gave the corresponding alcohol with no diastereo-



Scheme 1. Hydrogenation of C17=C18 in porphyrins to C17H-C18H in chlorins. (a) Stereoselective enzymatic *anti*-reduction of protochlorophyllide-*a* to (17*S*,18*S*)-chlorophyllide-*a*: R³ = CH=CH₂, E = COOCH₃, R = H, M = Mg; (b) non-stereoselective photoinduced *syn*-reduction of zinc protoChl derivatives to (17*R*,18*S*)- and (17*S*,18*R*)-Zn-Chl derivatives: R³ = CH₂CH₃ or CH₂OH, E = H, R = CH₃, M = Zn.

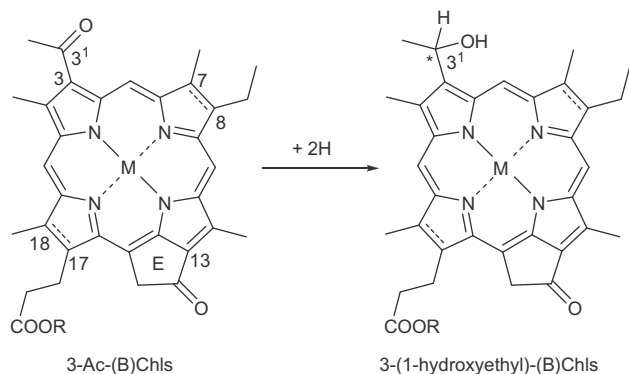
meric control.^{3,5} The neighboring C7 stereogenic center did not affect the reduction of the 3-Ac moiety: that is, 1,6-induction through the five bonds. The lack of asymmetric reduction of the 3-acetyl group in the above chiral (B)Chls with an achiral reagent would be ascribable to free rotation of the C3-C3¹ bond enabling it to take two conformers (Fig. 1)⁶ where the 3-Ac was coplanar with the cyclic tetrapyrrole π -system.

All photosynthetically active (B)Chls have a keto-carbonyl group at the 13-position and the oxo moiety located on the five-membered exo-ring (E-ring, see the left drawing of Scheme 2).⁷ The 13-C=O is more π -conjugated with the tetrapyrrole ring and

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Scheme 2. Reduction of 3-acetyl-(B)Chl derivatives to 3-(1-hydroxyethyl)-(B)Chls analogues. (a) Stereoselective reduction with a chiral reagent: R = CH₃, M = H₂, C7=C8/C17=C18, C7=C8/C17S–C18S or C7R–C8R/C17S–C18S; (b) non-stereoselective reduction with achiral NaBH₄: R = CH₃ or phytol, M = H₂ or Mg, C7=C8/C17S–C18S (for a Chl-*a* derivative) or C7R–C8R/C17S–C18S (for a BChl-*a* derivative). One of the naturally occurring BChls-*d* is drawn in the right structure with R = farnesyl, M = Mg and C7=C8/C17S–C18S.

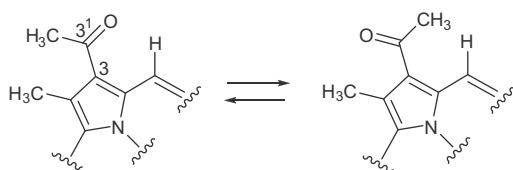
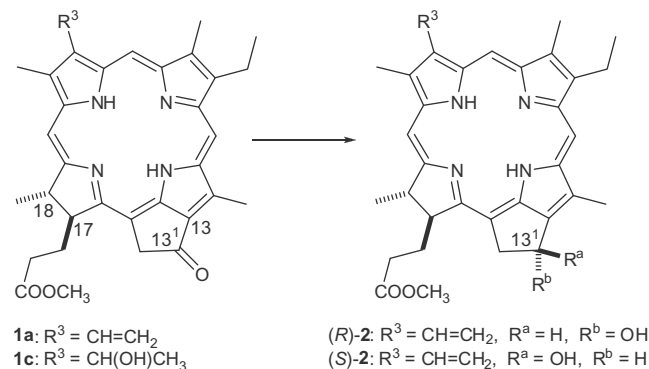


Fig. 1. Conformers (rotamers) around the 3-acetyl group in (B)Chl derivatives.

less reactive than the 3-C=O.^{8,9} Prolonged reactions or harsh conditions could modify the 13-C=O group to the corresponding alcohol, 13-CX(OH).¹⁰ Methyl pyropheophorbide-*a* **1a** was typically reduced in a solution with sodium borohydride (NaBH₄) to give a 13¹-epimeric mixture of (*R*)-**2** and (*S*)-**2** (Scheme 3).^{11–15} The stereoselectivity was reported to be nearly zero to 20% diastereomeric excess (de) and slightly more (*S*)-**2** was produced than (*R*)-**2**. The small but apparent diastereomeric control was ascribable to conformational restriction of the 13-C=O group: a 1,5-induction through the four bonds between C13¹ and C17S. Moreover, a similar asymmetric methylation of the 13-C=O as well as its reduction were observed in methyl bacteriopheophorbide-*d* **1c**.¹⁶

Herein we report the stereoselective reduction, methylation, and phenylation of the 13-carbonyl group in Chl-*a* derivatives **1** bearing (17S,18S)-stereogenic centers with achiral reagents in a solution. The resulting alcohols were epimeric mixtures and easily separated by conventional reverse phase (RP)-HPLC while the ste-



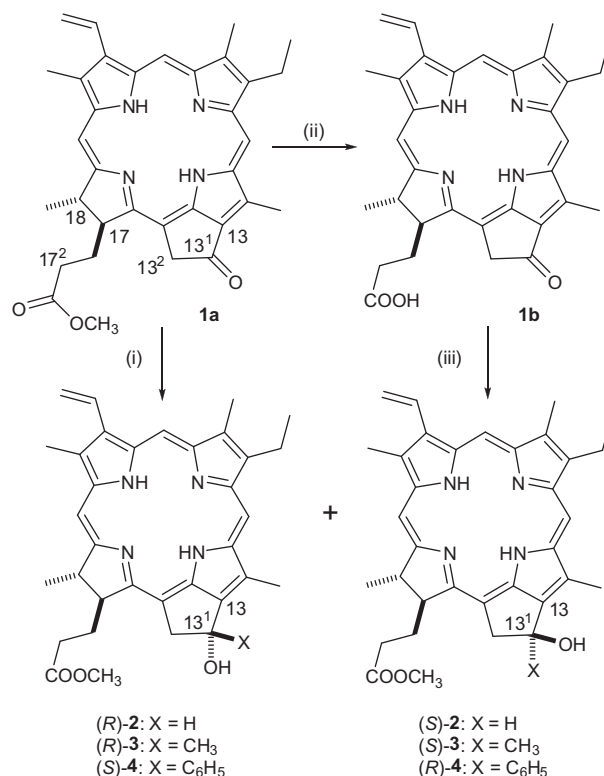
Scheme 3. Reaction of the 13-C=O in (B)Chl derivatives **1** to 13¹-hydroxy-chlorins.

reochemistry of the diastereomers was determined by two-dimensional (2D) NMR spectroscopy. The diastereomeric control is also discussed with regard to the steric effect in the molecule.

2. Results and discussion

2.1. Stereoselective reduction of the 13-carbonyl group in methyl pyropheophorbide-*a* **1a**

Methyl pyropheophorbide-*a* **1a** has two carbonyl groups at the 13- and 17²-positions (see the left upper drawing of Scheme 4). The former keto-carbonyl group was more reactive than the latter ester-carbonyl group. Conventional reduction by NaBH₄ occurred regioselectively at the 13-C=O moiety to give methyl 13¹-deoxo-13¹-hydroxy-pyropheophorbide-*a* **2** as the product. The carbinol obtained was a 13¹-epimeric mixture (see the lower drawing of Scheme 4, X = H) and the *de* values were reported to be 0¹⁴ or 10–20%.¹³ Here we re-examined the reduction of **1a** in dichloromethane (CH₂Cl₂) with NaBH₄ in methanol (MeOH) at room temperature. The ¹H NMR spectra of product **2** purified by silica gel chromatography showed the ratio to be 1:1.7. Analysis by RP-HPLC supported this ratio (Fig. 2a). The present reduction proceeded in 25 ± 3% *de* (from 5 independent experiments), which was slightly larger than the reported values. The stereoisomers were easily separated by RP-HPLC; the fast (major) and slow eluting (minor) bands are called **2#1** and **2#2**, respectively; the separation ratio (*R_s*) was 2.4. The isolated epimers were analyzed by 1D- and 2D-¹H NMR including ¹H-¹H COSY and NOESY in deuterated chloroform (CDCl₃) at room temperature. The stereochemistry at the 17-stereogenic position of both the epimers was fixed in the (*S*)-configuration. Since the 17-H was located near the 13²-H^b and removed from the 13²-H^a, both the 13²-H^a and H^b signals were



Scheme 4. Reaction of the 13-C=O in pyropheophorbides **1** to carbinols (*R/S*)-**2**-**4**. Reagents and conditions: (i) NaBH₄/CH₂Cl₂-MeOH or *l*-selectride/THF, rt; (ii) H₂SO₄/H₂O-THF; (iii) CH₃Li/Et₂O or C₆H₅Li/Bu₂O, TMEDA/THF, -40 or -60 °C to rt and CH₂N₂/Et₂O.

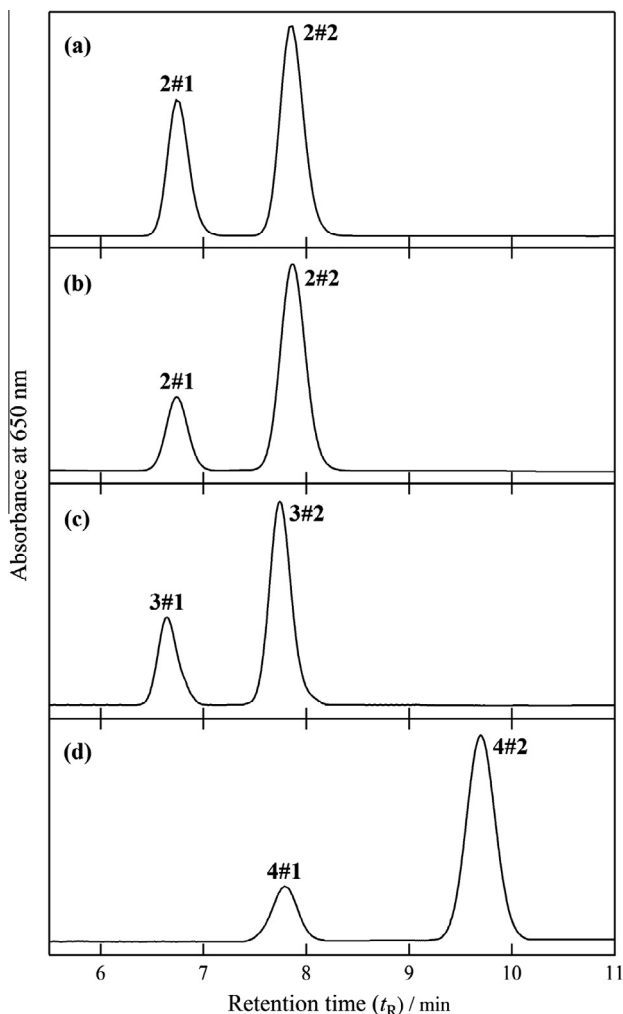


Fig. 2. RP-HPLC of (a) **2#1/2** (= *(R/S)*-**2**, by NaBH_4), (b) **2#1/2** (= *(R/S)*-**2**, by *L*-selectride), (c) **3#1/2** (= *(R/S)*-**3**), and (d) **4#1/2** (= *(R/S)*-**4**). Conditions: Cosmosil 5C₁₈-AR-II (4.6 mm ϕ \times 150 mm), MeOH/H₂O = 95:5 (v/v), and 1.0 mL/min.

assigned from their NOESY spectra (Fig. 3). The NOESY spectrum of **2#1** showed that its 13¹-H was correlated with the 13²-H^a but not with 13²-H^b (Fig. 3a). The stereochemistry at the 13¹-position of **2#1** was confirmed to have an (*R*)-configuration: **2#1** = (*R*)-**2**. Based on the NOE-correlation of 13¹-H with 13²-H^b (Fig. 3b), the major fraction **2#2** was assigned as (*S*)-**2**.

In one of the reduced products, (*R*)-**2**, the hydroxy group at the 13¹-position was located in the same direction as the methyl group at the 18-position based on the chlorin π -plane, and in the reverse

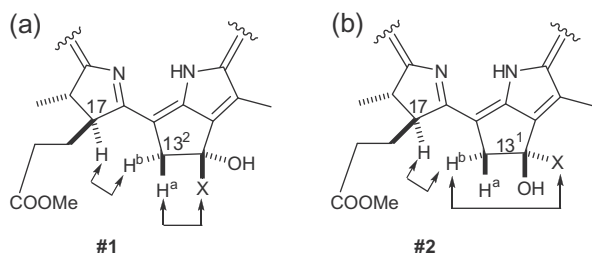


Fig. 3. Specific NOE correlations among the 13¹-X^a/X^b, 13²-H^a/H^b and 17-H in (a) the first and minor fractions, **2#1** (= (*R*)-**2**) (X = H), **3#1** (= (*R*)-**3**) (X = CH₃), and **4#1** (= (*S*)-**4**) (X = C₆H₅, *ortho*-H); (b) the second and major fractions, **2#2** (= (*S*)-**2**) (X = H), **3#2** (= (*S*)-**3**) (X = CH₃), and **4#2** (= (*R*)-**4**) (X = C₆H₅, *ortho*-H).

of the propionate residue at the 17-position. The two polar substituents, 13¹-OH and 17²-COOMe, gave an *anti*-configuration in (*R*)-**2**. The other epimer, (*S*)-**2** had the *syn*-configuration for the two substituents and was less hydrophilic than (*R*)-**2**. Therefore, the relatively hydrophilic (*R*)-**2** eluted more rapidly in RP-HPLC than the relatively hydrophobic (*S*)-**2**. The partition coefficient between the 1-octanol and aqueous phases (P) was calculated from a molecular structure and the ClogP values of (*R*)-**2** and (*S*)-**2** were 8.6 and 10.1, respectively. The difference showed that (*R*)-**2** was more hydrophilic than (*S*)-**2**. The elution order supports the above structural assignments of the separated epimers.

The 13-carbonyl group of **1a** has two faces; the front and back faces shown in Scheme 4 are the *si*- and *re*-faces. A hydride attacked the 13-C=O moiety from the *si*- and *re*-face to give 13¹-epimers (*R*)-**2** and (*S*)-**2**, respectively. The *si*-face was sterically crowded with the 17-propionate residue, while the *re*-face was hindered less by the small and far 18-methyl group. During the present reduction, the H[−] of NaBH₄ favorably attacked the partially positively-charged 13¹-carbon atom from the less sterically demanding *re*-face and more (*S*)-**2** was produced than (*R*)-**2** (see also the Graphical abstract). It should be noted that no epimerization between (*R*)-**2** and (*S*)-**2** was observed during the reduction as well as their purification and analysis.

Lithium tri(*sec*-butyl)borohydride (*L*-selectride) is known to be a sterically larger reductant than NaBH₄.¹⁷ Treatment of **1a** with a tetrahydrofuran (THF) solution of *L*-selectride gave an epimeric mixture of (*R*)-**2** and (*S*)-**2** as the products. The ¹H NMR and HPLC analyses of the mixture showed that (*S*)-**2** was produced in a 3.4-fold higher yield than (*R*)-**2** (Fig. 2b). The (*S*)-preference was the same as in the reduction by NaBH₄. The *de* value in the reduction by an *L*-selectride was 55% and approximately two fold larger than that by NaBH₄. When the hydride of a large *L*-selectride reacted with the 13-C=O of **1a**, the reagent greatly interacted with the 17-propionate residue and attacked the C13¹-atom from the *re*-face to favorably give (*S*)-**2**. These results indicate that steric factors affected the stereoselective reduction.

2.2. Stereoselective methylation of the 13-carbonyl group in pyropheophorbide-*a* **1b**

Methylation of the 13-carbonyl group in **1a** was also examined. The Grignard reaction of **1a** with CH₃MgI gave complex products containing methylated tertiary alcohol **3**, since the reagent was so reactive.¹⁸ The methyl Grignard reagent partially attacked the 13-C=O and also abstracted the proton on the 13²-carbon. The former gave the desired product **3** while the latter afforded a cyclized product through intramolecular Claisen-type reaction with the 17²-COOMe. A similar situation has already been observed.⁹ In order to suppress the undesired cyclization, the methyl ester of **1a** was first hydrolyzed and the resulting carboxylic acid **1b** was used for the methylation of the 13-C=O group. Treatment of **1b** in THF with methyl lithium (CH₃Li) in diethyl ether (Et₂O) in the presence of *N,N,N',N'*-tetramethylethylenediamine (TMEDA) at −40 °C⁹ first produced the lithium carboxylate (17²-COOLi) and then the 13¹-methylated compound [13-CCH₃(OLi)]. The initially produced salt could not be reacted with the 13²-anion due to the negative charge on the 17²-COO[−]. Double protonation of the product followed by methyl esterification gave an epimeric mixture of (*R*)-**3** and (*S*)-**3**. The tertiary alcohol obtained was slightly unstable on silica gel; thus alumina column chromatography was necessary for its purification.

The epimeric mixture of (*R/S*)-**3** was fully separated by RP-HPLC (Fig. 2c, *R_s* = 2.4) and the first and second fractions **3#1/2** were analyzed by ¹H NMR. Using the NOE correlations of the 13¹-CH₃ with 13²-H, **3#1** and **3#2** were assigned as (*R*)-**3** and (*S*)-**3**, respectively. The elution order was consistent with that of **2**. The ClogP values of

(*R*)-**3** and (*S*)-**3** were 8.7 and 10.7, respectively; (*R*)-**3** should be more hydrophilic than (*S*)-**3**, thus supporting the elution order.

Figure 2c shows that the ratio of (*R*)-**3** and (*S*)-**3** was 1:2.3. The 13^1 -epimer (*S*)-**3** produced by the methylation from the sterically less crowded *re*-face of **1b** was the major product, similar to the above reduction. The stereoselectivity (ca. 40% de) was higher than that in the reduction by NaBH₄. This enhancement could be due to the finding that the methylating reagent (a methyl anion) was bulkier than the reducing reagent (hydride) in NaBH₄, although the effect of the hydrolysis of methyl ester **1a** to the carboxylic acid **1b** cannot be ruled out. It is noteworthy that no epimerization at the 3^1 -position occurred during purification and that the separated epimer (*R*)-**3** could not be isomerized to (*S*)-**3** after standing in its aqueous MeOH solution at room temperature for one week and vice versa.

2.3. Stereoselective phenylation of the 13-carbonyl group in pyropheophorbide-*a* **1b**

The 13-C=O in **1b** was phenylated similarly to the aforementioned methylation. After methyl-esterification, product **4** was obtained as a 13^1 -epimeric mixture. The isolated yield for the two steps of **1b** to **4** was 7% and was lower than that of **1b** to **3** (33%). The esterification of the carboxylic acid with diazomethane was achieved almost quantitatively. The inefficiency in the phenylation was partially due to the steric bulkiness in phenyl lithium (C₆H₅Li).

The epimeric mixture **4** was readily separated by RP-HPLC (Fig. 2d, *R_s* = 3.4) and the stereochemistry of **4#1/2** was determined by their ¹H NMR spectra to be (*S*)-**4** and (*R*)-**4**, respectively. It should be noted that the relative stereochemistry at the 13^1 -stereogenic center of (*S*)-**4** is the same as those of (*R*)-**2** and (*R*)-**3** while that of (*R*)-**4** is consistent with those of (*S*)-**2** and (*S*)-**3**, although the (13^1 *R/S*)-nomenclature is changed by X = H/CH₃ to C₆H₅ (Scheme 4). The phenylation from the *si*- and *re*-faces of **1b** led to the formation of (*S*)-**4** and (*R*)-**4**, respectively. Similar to the reduction and methylation, the alcohol produced via attack of a phenyl anion from the *re*-face was the major product, (*R*)-**4** and eluted more slowly in RP-HPLC: Clog*P* = 10.5 for (*S*)-**4** < 11.9 for (*R*)-**4**. The (*R*)-enriched product was obtained in approximately 65% de. The highest stereoselectivity in the reactions examined herein is due to the sterically bulky phenylation reagent. No epimerization at the 13^1 -position of either (*R*)-**4** or (*S*)-**4** was observed in an aqueous MeOH solution.

3. Conclusion

Diastereo-face differentiation was achieved in the reduction, methylation, and phenylation of the conformationally fixed 13-C=O group in pyropheophorbides-*a*. The *si*-face was sterically crowded by the neighboring 17-propionate residue and the major carbinols were produced by the attack of reactants from the *re*-face. The stereoselectivity was controlled by the steric interactions of the remote and four-bond separated substituent with the reactive species. The diastereomers produced can be easily separated by RP-HPLC due to the difference of their hydrophobicity/hydrophilicity and their stereochemistry was confirmed by their ¹H-¹H NOE correlations and HPLC elution order. Their Clog*P* values were useful for the estimation of the HPLC retention times (*t_R*) for the diastereomers (see Fig. 4).

4. Experimental

4.1. General

Visible absorption spectra were measured with a Hitachi U-3500 spectrophotometer. 1D- or 2D-¹H NMR spectra were

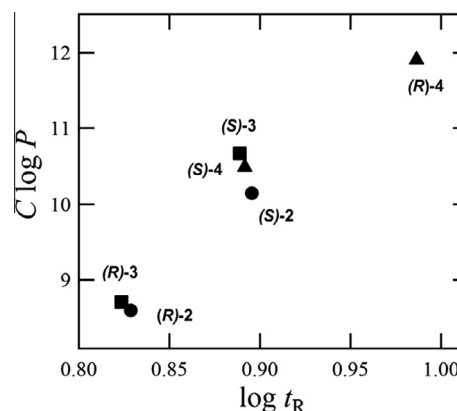


Fig. 4. Correlation between the *t_R* (min) and Clog*P* of diastereomers (*R*)- and (*S*)-**2-4**: see Section 4.1 for the RP-HPLC separation conditions.

recorded on a JEOL AL-400 (400 MHz) or ECA-600 (600 MHz) spectrometer, respectively; CHCl₃ (δ_{H} = 7.26 ppm) was used as an internal reference. Time-of-flight (TOF) mass data were obtained using direct laser desorption/ionization by a Shimadzu AXIMA-CFR plus spectrometer. Fast atomic bombardment (FAB)-MS spectra were measured with a JEOL GCmate II spectrometer; FAB-MS samples were dissolved in CH₂Cl₂, *m*-nitrobenzyl alcohol was used as the matrix and PEG600 was added as an internal reference for high resolution mass measurements. Silica gel or alumina column chromatography was performed with Kieselgel 60 (Merck, 40–63 μ m, 230–400 mesh) or aluminum oxide 90 (activity I, neutral, Merck, 63–200 μ m, 70–230 mesh) deactivated by 10% (v/v) H₂O, respectively. RP-HPLC was done with a Shimadzu LC-10ADvp pump and SPD-M10Avp photodiode-array detector: Cosmosil 5C₁₈-AR-II (4.6 mm ϕ \times 150 mm) as an ODS column and MeOH/H₂O = 95:5 (1.0 mL/min) as an eluent. Clog*P* values were calculated by a ChemDraw Ultra software (version 8.0.3).

All reactions were carried out in the dark. Methyl pyropheophorbide-*a* **1a** and pyropheophorbide-*a* **1b** were prepared according to the literature.¹⁹ Commercially available CH₂Cl₂, MeOH, and THF (Nacalai Tesque) were used as the reaction solvents. THF was distilled over calcium hydride just before use. NaBH₄ and TMEDA were purchased from Wako Pure Chemical Ind. and Nacalai Tesque, respectively. *l*-Selectride [LiBH(CHMeEt)₃] in THF (ca. 1 M), CH₃Li in Et₂O (1.14 M), and C₆H₅Li in dibutyl ether (Bu₂O) (1.9 M) were obtained from Tokyo Chemical Ind., Kanto Chemical, and Aldrich, respectively.

4.2. Modification of the 13-carbonyl group in methyl pyropheophorbide-*a*

4.2.1. Synthesis of methyl 13^1 -deoxo- 13^1 -hydroxy-pyropheophorbide-*a* **2**

Ketone **1a** (51.2 mg, 93.4 μ mol) was dissolved in CH₂Cl₂ (20 mL), to which NaBH₄ (80.9 mg, 2.13 mmol) and MeOH (2 mL) were added. The mixture was stirred under N₂ at room temperature for approximately 1 h. After the disappearance of **1a** (monitoring the visible spectrum in a solution: the shift of Qy peaks from 668 to 653 nm), the reaction was quenched with aq 2% HCl and stirred for 10 min. The separated organic phase was washed with aq 4% NaHCO₃ and H₂O, dried over Na₂SO₄, and filtered. After evaporation, the residue was purified by silica gel column chromatography (CH₂Cl₂) to give alcohol **2** (29.5 mg, 53.6 μ mol, 57%) as a 1:1.7 mixture of (13^1 *R*)- and (13^1 *S*)-epimers: Vis (CH₂Cl₂) λ_{max} = 652 (relative absorbance, 0.27), 597 (0.03), 546 (0.01), 502 (0.09), 401 nm (1.00); ¹H NMR (400 MHz, CDCl₃) δ (13^1 *R/S* = 1/1.7) = 9.88 (1H, s, 5-H), 9.65 (1H, s, 10-H), 8.91 (1H, s, 20-H), 8.24

(1H, dd, $J = 12, 18$ Hz, 3-CH), 6.53 (1H, m, 13-CH), 6.35 (1H, dd, $J = 2, 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.18 (1H, dd, $J = 2, 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.41/5.31 (1H, dd, $J = 6, 16$ Hz, 13¹-CH *cis* to 13-C-H), 4.62/4.76 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-H), 4.65 (1H, q, $J = 7$ Hz, 18-H), 4.46 (1H, m, 17-H), 3.85 (2H, q, $J = 8$ Hz, 8-CH₂), 3.63 (3H, s, 12-CH₃), 3.57 (3H, s, 2-CH₃), 3.57/3.43 (3H, s, 17²-COOCH₃), 3.42 (3H, s, 7-CH₃), 2.81–2.74, 2.62–2.53, 2.45–2.31, 2.25–2.17 (each 1H, m, 17-CH₂CH₂), 1.85 (3H, d, $J = 7$ Hz, 18-CH₃), 1.76 (3H, t, $J = 8$ Hz, 8¹-CH₃), –1.37, –3.22 (each 1H, s, NH $\times 2$); MS (TOF) found: m/z 550.0. Calcd for C₃₄H₃₈N₄O₃: M⁺, 550.3. Please also see the spectroscopic data in the literature.^{12,13}

The above diastereomeric mixture was separated by RP-HPLC to afford (13¹R)-epimer (R)-**2** at $t_R = 6.7$ min and (13¹S)-epimer (S)-**2** at 7.9 min. (R)-**2**: Vis (CH₂Cl₂) $\lambda_{\max} = 653$ (relative absorbance, 0.26), 599 (0.03), 544 (0.03), 502 (0.10), 402 nm (1.00); ¹H NMR (400 MHz, CDCl₃) $\delta = 9.88$ (1H, s, 5-H), 9.65 (1H, s, 10-H), 8.91 (1H, s, 20-H), 8.23 (1H, dd, $J = 12, 18$ Hz, 3-CH), 6.56 (1H, d, $J = 6$ Hz, 13-CH), 6.35 (1H, dd, $J = 1, 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.18 (1H, dd, $J = 1, 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.41 (1H, dd, $J = 6, 16$ Hz, 13¹-CH *cis* to 13-C-H), 4.65 (2H, q, $J = 7.5$ Hz, 18-H), 4.63 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-H), 4.47 (1H, d, $J = 9$ Hz, 17-H), 3.85 (2H, q, $J = 7.5$ Hz, 8-CH₂), 3.63 (3H, s, 12-CH₃), 3.571 (3H, s, 17²-COOCH₃), 3.565 (3H, s, 2-CH₃), 3.42 (3H, s, 7-CH₃), 2.78–2.71, 2.62–2.54, 2.37–2.31, 2.24–2.18 (each 1H, m, 17-CH₂CH₂), 1.85 (3H, d, $J = 7.5$ Hz, 18-CH₃), 1.77 (3H, t, $J = 7.5$ Hz, 8¹-CH₃), –1.34, –3.20 (each 1H, s, NH $\times 2$); MS (TOF) found: m/z 550.6. Calcd for C₃₄H₃₈N₄O₃: M⁺, 550.3. (S)-**2**: Vis (CH₂Cl₂) $\lambda_{\max} = 652$ (relative absorbance, 0.27), 597 (0.03), 549 (0.01), 502 (0.10), 401 nm (1.00); ¹H NMR (400 MHz, CDCl₃) $\delta = 9.88$ (1H, s, 5-H), 9.65 (1H, s, 10-H), 8.91 (1H, s, 20-H), 8.24 (1H, dd, $J = 12, 18$ Hz, 3-CH), 6.52 (1H, d, $J = 6$ Hz, 13-CH), 6.35 (1H, dd, $J = 2, 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.18 (1H, dd, $J = 2, 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.30 (1H, dd, $J = 6, 16$ Hz, 13¹-CH *cis* to 13-C-H), 4.75 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-H), 4.65 (1H, q, $J = 7.5$ Hz, 18-H), 4.45 (1H, d, $J = 9$ Hz, 17-H), 3.85 (2H, q, $J = 7.5$ Hz, 8-CH₂), 3.63 (3H, s, 12-CH₃), 3.57 (3H, s, 2-CH₃), 3.421 (3H, s, 17²-COOCH₃), 3.418 (3H, s, 17-CH₃), 2.81–2.74, 2.61–2.53, 2.45–2.36, 2.24–2.17 (each 1H, m, 17-CH₂CH₂), 1.86 (3H, d, $J = 7.5$ Hz, 18-CH₃), 1.77 (3H, t, $J = 7.5$ Hz, 8¹-CH₃), –1.37, –3.32 (each 1H, s, NH $\times 2$); MS (TOF) found: m/z 550.6. Calcd for C₃₄H₃₈N₄O₃: M⁺, 550.3. Please also see the spectroscopic data in the literature.¹⁵

Similar to the above synthesis, the reduction of **1a** (54.0 mg, 98.5 μ mol) in THF (5 mL) with *l*-selectride in THF (1 M, 100 μ L, 100 μ mol) for 30 min gave a 1:3.4 mixture of (R)-**2** and (S)-**2** (10.9 mg, 19.8 μ mol, 20%) and **1a** (42.6 mg, 77.7 μ mol) was recovered. The yield based on consumed **1a** was 95%. Using 5 equiv of *l*-selectride, **1a** was completely reduced to afford the same 1:3.4 mixture of (R)-**2**/(S)-**2**, while by-products bearing a 17-hydroxypropyl group were partially produced through further reduction of the 17²-COOMe to CH₂OH.

4.2.2. Synthesis of methyl 13¹-deoxo-13¹-hydroxy-13¹-methylpyropheophoride-a **3**

Ketone **1b** (91.2 mg, 171 μ mol) was dissolved in THF (40 mL) at –40 °C, to which TMEDA (2 mL, 13.4 mmol) and CH₃Li in Et₂O (1.14 M, 6.00 mL, 6.84 mmol) were added. The mixture was stirred under Ar at –40 °C until the disappearance of **1b** (monitoring the visible spectrum in a solution: the shift of Qy peaks from 668 to 653 nm) and quenched with aq 2% HCl (10 mL) at –40 °C. The solution was diluted with CHCl₃ and the separated organic phase was washed with aq 4% NaHCO₃ and H₂O, dried over Na₂SO₄, and filtered. After evaporation, the residue was dissolved in THF (40 mL) and stirred with an excess amount of CH₂N₂ in Et₂O at room temperature for 15 min. All the solvents were distilled *in vacuo* and the residue was purified by alumina column chromatography (CH₂Cl₂/hexane = 1:3) to give methyl carbinol **3** (32.0 mg,

56.7 μ mol, 33%) as a 1:2.3 mixture of (13¹R)- and (13¹S)-epimers: Vis (CH₂Cl₂) $\lambda_{\max} = 652$ (relative absorbance, 0.28), 596 (0.03), 502 (0.09), 402 nm (1.00); ¹H NMR (400 MHz, CDCl₃) δ (13¹R/S = 1/2.3) = 9.88 (1H, s, 5-H), 9.63 (1H, s, 10-H), 8.93 (1H, s, 20-H), 8.23 (1H, dd, $J = 12, 18$ Hz, 3-CH), 6.35 (1H, d, $J = 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.18 (1H, d, $J = 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.07/5.03 (1H, d, $J = 16$ Hz, 13¹-CH *cis* to 13-C-CH₃), 4.84/4.94 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-CH₃), 4.64 (1H, q, $J = 7$ Hz, 18-H), 4.43 (1H, d, $J = 9$ Hz, 17-H), 3.86 (2H, q, $J = 8$ Hz, 8-CH₂), 3.62 (3H, s, 12-CH₃), 3.59/3.38 (3H, s, 17²-COOCH₃), 3.57 (3H, s, 2-CH₃), 3.42 (3H, s, 7-CH₃), 2.75–2.71, 2.59–2.48, 2.41–2.33, 2.30–2.23 (each 1H, m, 17-CH₂CH₂), 2.30/2.20 (3H, s, 13¹-CH₃), 1.87 (3H, d, $J = 7$ Hz, 18-CH₃), 1.78 (3H, t, $J = 8$ Hz, 8¹-CH₃), –1.47, –3.24 (each 1H, s, NH $\times 2$); MS (TOF) found: m/z 565.8. Calcd for C₃₅H₄₁N₄O₃: M⁺, 565.3; HRMS (FAB) found: m/z 565.3176. Calcd for C₃₅H₄₁N₄O₃: M⁺, 565.3179.

The above diastereomeric mixture was separated by RP-HPLC to afford (13¹R)-epimer (R)-**3** at $t_R = 6.7$ min and (13¹S)-epimer (S)-**3** at 7.7 min. (R)-**3**: Vis (CH₂Cl₂) $\lambda_{\max} = 653$ (relative absorbance, 0.27), 597 (0.03), 502 (0.10), 402 nm (1.00); ¹H NMR (400 MHz, CDCl₃) $\delta = 9.88$ (1H, s, 5-H), 9.64 (1H, s, 10-H), 8.91 (1H, s, 20-H), 8.25 (1H, dd, $J = 12, 18$ Hz, 3-CH), 6.35 (1H, dd, $J = 1.5, 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.18 (1H, dd, $J = 1.5, 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.12 (1H, d, $J = 16$ Hz, 13¹-CH *cis* to 13-C-CH₃), 4.90 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-CH₃), 4.65 (2H, q, $J = 7.5$ Hz, 18-H), 4.44 (1H, d, $J = 9$ Hz, 17-H), 3.85 (2H, q, $J = 7.5$ Hz, 8-CH₂), 3.65 (3H, s, 12-CH₃), 3.58 (3H, s, 17²-COOCH₃), 3.57 (3H, s, 2-CH₃), 3.42 (3H, s, 7-CH₃), 2.77–2.71, 2.36–2.22, (each 1H, m, 17-CH₂), 2.63–2.57, 2.24–2.18 (each 1H, m, 17¹-CH₂), 2.42 (3H, s, 13¹-CH₃), 1.84 (3H, d, $J = 7.5$ Hz, 18-CH₃), 1.76 (3H, t, $J = 7.5$ Hz, 8¹-CH₃), –3.22 (1H, s, NH) [another NH could not be observed.]; MS (TOF) found: m/z 564.5. Calcd for C₃₅H₄₀N₄O₃: M⁺, 564.3; HRMS (FAB) found: m/z 565.3180. Calcd for C₃₅H₄₁N₄O₃: M⁺, 565.3179. (S)-**3**: Vis (CH₂Cl₂) $\lambda_{\max} = 652$ (relative absorbance, 0.28), 597 (0.03), 503 (0.09), 402 nm (1.00); ¹H NMR (400 MHz, CDCl₃) $\delta = 9.89$ (1H, s, 5-H), 9.64 (1H, s, 10-H), 8.91 (1H, s, 20-H), 8.24 (1H, dd, $J = 12, 18$ Hz, 3-CH), 6.35 (1H, dd, $J = 1.5, 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.18 (1H, dd, $J = 1.5, 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.07 (1H, d, $J = 16$ Hz, 13¹-CH *cis* to 13-C-CH₃), 4.99 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-CH₃), 4.64 (1H, q, $J = 7.5$ Hz, 18-H), 4.45 (1H, d, $J = 9$ Hz, 17-H), 3.85 (2H, q, $J = 7.5$ Hz, 8-CH₂), 3.65 (3H, s, 12-CH₃), 3.57 (3H, s, 2-CH₃), 3.42 (3H, s, 7-CH₃), 3.38 (3H, s, 17²-COOCH₃), 2.80–2.72, 2.44–2.37, (each 1H, m, 17-CH₂), 2.59–2.51, 2.23–2.15 (each 1H, m, 17¹-CH₂), 2.32 (3H, s, 13¹-CH₃), 1.87 (3H, d, $J = 7.5$ Hz, 18-CH₃), 1.76 (3H, t, $J = 7.5$ Hz, 8¹-CH₃), –1.17, –3.01 (each 1H, s, NH $\times 2$); MS (TOF) found: m/z 564.6. Calcd for C₃₅H₄₀N₄O₃: M⁺, 564.3; HRMS (FAB) found: m/z 565.3179. Calcd for C₃₅H₄₁N₄O₃: M⁺, 565.3179.

4.2.3. Synthesis of methyl 13¹-deoxo-13¹-hydroxy-13¹-phenylpyropheophoride-a **4**

Similar to the synthesis of **3**, the reaction of **1b** (105 mg, 197 μ mol) with C₆H₅Li in Bu₂O (1.9 M, 10 mL, 19 mmol) at –60 °C to room temperature gave phenyl carbinol **4** (8.4 mg, 13.4 μ mol, 7%) as a 1:4.5 mixture of (13¹S)- and (13¹R)-epimers: Vis (CH₂Cl₂) $\lambda_{\max} = 653$ (relative absorbance, 0.29), 598 (0.03), 502 (0.10), 402 nm (1.00); ¹H NMR (400 MHz, CDCl₃) δ (13¹S/R = 1/4.5) = 9.89 (1H, s, 5-H), 9.60 (1H, s, 10-H), 8.91 (1H, s, 20-H), 8.25 (1H, dd, $J = 12, 18$ Hz, 3-CH), 7.82/7.69 (2H, d, $J = 7.5$ Hz, *o*-H of 13¹-Ph), 7.41/7.38 (2H, t, $J = 7.5$ Hz, *m*-H of 13¹-Ph), 7.38/7.32 (1H, t, $J = 7.5$ Hz, *p*-H of 13¹-Ph), 6.37 (1H, d, $J = 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.19 (1H, d, $J = 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.31/5.24 (1H, d, $J = 16$ Hz, 13¹-CH *cis* to 13-C-Ph), 5.18/5.37 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-Ph), 4.64

(1H, q, $J = 7$ Hz, 18-H), 4.40 (1H, d, $J = 8$ Hz, 17-H), 3.84 (2H, q, $J = 8$ Hz, 8-CH₂), 3.58 (3H, s, 2-CH₃), 3.55/3.31 (3H, s, 17²-COOCH₃), 3.42 (3H, s, 7-CH₃), 3.39 (3H, s, 12-CH₃), 2.79–2.74, 2.57–2.51, 2.48–2.43, 2.20–2.15 (each 1H, m, 17-CH₂CH₂), 1.86 (3H, d, $J = 7$ Hz, 18-CH₃), 1.76 (3H, t, $J = 8$ Hz, 8¹-CH₃), –1.47, –3.24 (each 1H, s, NH \times 2); MS (TOF) found: m/z 626.7. Calcd for C₄₀H₄₂N₄O₃: M⁺, 626.3; HRMS (FAB) found: m/z 627.3315. Calcd for C₄₀H₄₃N₄O₃: MH⁺, 627.3335.

The above diastereomeric mixture was separated by RP-HPLC to afford (13¹S)-epimer (S)-**4** at $t_R = 7.8$ min and (13¹R)-epimer (R)-**4** at 9.7 min. (S)-**4**: Vis (CH₂Cl₂) $\lambda_{\max} = 653$ (relative absorbance, 0.29), 598 (0.03), 502 (0.09), 402 nm (1.00); ¹H NMR (400 MHz, CDCl₃) $\delta = 9.88$ (1H, s, 5-H), 9.65 (1H, s, 10-H), 8.92 (1H, s, 20-H), 8.24 (1H, dd, $J = 12$, 18 Hz, 3-CH), 7.82 (2H, d, $J = 7.5$ Hz, o-H of 13¹-Ph), 7.46 (2H, t, $J = 7.5$ Hz, m-H of 13¹-Ph), 7.38 (1H, t, $J = 7.5$ Hz, p-H of 13¹-Ph), 6.35 (1H, dd, $J = 1.5$, 18 Hz, 3¹-CH *trans* to 3-C-H), 6.19 (1H, dd, $J = 1.5$, 12 Hz, 3¹-CH *cis* to 3-C-H), 5.34 (1H, d, $J = 16$ Hz, 13¹-CH *cis* to 13-C-Ph), 5.18 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-Ph), 4.66 (2H, q, $J = 7.5$ Hz, 18-H), 4.45 (1H, d, $J = 9$ Hz, 17-H), 3.83 (2H, q, $J = 7.5$ Hz, 8-CH₂), 3.57 (3H, s, 2-CH₃), 3.55 (3H, s, 17²-COOCH₃), 3.42 (3H, s, 7-CH₃), 3.34 (3H, s, 12-CH₃), 2.73–2.71, 2.25–2.20, (each 1H, m, 17-CH₂), 2.62–2.54, 2.31–2.26 (each 1H, m, 17¹-CH₂), 1.85 (3H, d, $J = 7.5$ Hz, 18-CH₃), 1.75 (3H, t, $J = 7.5$ Hz, 8¹-CH₃), –1.25, –3.22 (each 1H, s, NH \times 2); MS (TOF) found: m/z 625.7. Calcd for C₄₀H₄₂N₄O₃: M⁺, 626.3; HRMS (FAB) found: m/z 626.3249. Calcd for C₄₀H₄₂N₄O₃: M⁺, 626.3257. (R)-**4**: Vis (CH₂Cl₂) $\lambda_{\max} = 653$ (relative absorbance, 0.29), 598 (0.03), 502 (0.09), 402 nm (1.00); ¹H NMR (400 MHz, CDCl₃) $\delta = 9.89$ (1H, s, 5-H), 9.66 (1H, s, 10-H), 8.92 (1H, s, 20-H), 8.25 (1H, dd, $J = 12$, 18 Hz, 3-CH), 7.69 (2H, d, $J = 7.5$ Hz, o-H of 13¹-Ph), 7.38 (2H, t, $J = 7.5$ Hz, m-H of 13¹-Ph), 7.32 (1H, t, $J = 7.5$ Hz, p-H of 13¹-Ph), 6.37 (1H, d, $J = 18$ Hz, 3¹-CH *trans* to 3-C-H), 6.19 (1H, d, $J = 12$ Hz, 3¹-CH *cis* to 3-C-H), 5.37 (1H, d, $J = 16$ Hz, 13¹-CH *trans* to 13-C-Ph), 5.24 (1H, d, $J = 16$ Hz, 13¹-CH *cis* to 13-C-Ph), 4.64 (1H, q, $J = 7.5$ Hz, 18-H), 4.40 (1H, d, $J = 9$ Hz, 17-H), 3.84 (2H, q, $J = 7.5$ Hz, 8-CH₂), 3.58 (3H, s, 2-CH₃), 3.42 (3H, s, 7-CH₃), 3.39 (3H, s, 12-CH₃), 3.30 (3H, s, 17²-COOCH₃), 2.80–2.72, 2.48–2.42, (each 1H, m, 17-CH₂), 2.54–2.50, 2.23–2.17 (each 1H, m, 17¹-CH₂), 1.86 (3H, d, $J = 7.5$ Hz, 18-CH₃), 1.76 (3H, t, $J = 7.5$ Hz, 8¹-CH₃), –1.27, –3.17 (each 1H, s, NH \times 2); MS (TOF) found: m/z 626.1. Calcd for C₄₀H₄₂N₄O₃: M⁺, 626.3; HRMS (FAB) found: m/z 626.3261. Calcd for C₄₀H₄₂N₄O₃: M⁺, 626.3257.

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References

- (a) Muraki, N.; Nomata, J.; Ebata, K.; Mizoguchi, T.; Shiba, T.; Tamiaki, H.; Kurisu, G.; Fujita, Y. *Nature* **2010**, *465*, 110–114; (b) Reinbothe, C.; Bakkouri, M. E.; Buhr, F.; Muraki, N.; Nomata, J.; Kurisu, G.; Fujita, Y.; Reinbothe, S. *Trends Plant Sci.* **2010**, *15*, 614–624; (c) Gabruk, M.; Grzyb, J.; Kruk, J.; Mysliwa-Kurdiel, B. *Photosynthetica* **2012**, *50*, 529–540; (d) Moser, J.; Lange, C.; Krausze, J.; Rebele, J.; Schubert, W.-D.; Ribbe, M. W.; Heinz, D. W.; Jahn, D. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 2094–2098.
- (a) Wolf, H.; Scheer, H. *Justus Liebigs Ann. Chem.* **1973**, 1710–1740; (b) Tamiaki, H.; Watanabe, T.; Kunieda, M. *Res. Chem. Int.* **2007**, *33*, 161–168.
- Tamiaki, H.; Kouraba, M.; Takeda, K.; Kondo, S.; Tanikaga, R. *Tetrahedron: Asymmetry* **1998**, *9*, 2101–2111.
- (a) Sasaki, S.; Omoda, M.; Tamiaki, H. *J. Photochem. Photobiol. A: Chem.* **2004**, *162*, 307–315; (b) Tamiaki, H.; Hamada, K.; Kunieda, M. *Tetrahedron* **2008**, *64*, 5721–5727; (c) Huber, V.; Sengupta, S.; Würthner, F. *Chem. Eur. J.* **2008**, *14*, 7791–7807.
- (a) Struck, A.; Cmiel, E.; Katheder, I.; Schäfer, W.; Scheer, H. *Biochim. Biophys. Acta* **1992**, *1101*, 321–328; (b) Kunieda, M.; Mizoguchi, T.; Tamiaki, H. *Photochem. Photobiol.* **2004**, *79*, 55–61.
- (a) Gudowska-Nowak, E.; Newton, M. D.; Fajer, J. *J. Phys. Chem.* **1990**, *94*, 5795–5801; (b) Tamiaki, H.; Kotegawa, Y.; Mizutani, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6037–6040.
- Tamiaki, H.; Shibata, R.; Mizoguchi, T. *Photochem. Photobiol.* **2007**, *83*, 152–162.
- Tamiaki, H.; Miyatake, T.; Tanikaga, R. *Tetrahedron Lett.* **1997**, *38*, 267–270.
- Yagai, S.; Miyatake, T.; Shimono, Y.; Tamiaki, H. *Photochem. Photobiol.* **2001**, *73*, 153–163.
- Tamiaki, H.; Kunieda, M. *Handb. Porphyr. Sci.* **2011**, *11*, 223–290.
- Wolf, H.; Scheer, H. *Justus Liebigs Ann. Chem.* **1971**, *745*, 87–98.
- Ma, L.; Dolphin, D. *Can. J. Chem.* **1997**, *75*, 262–275; Katterle, M.; Holzwarth, A. R.; Jesorka, A. *Eur. J. Org. Chem.* **2006**, 414–422.
- (a) Maruyama, K.; Yamada, H.; Osuka, A. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 3462–3466; (b) Tamiaki, H.; Watanabe, T.; Miyatake, T. *J. Porphyr. Phthalocya.* **1999**, *3*, 45–52.
- Jesorka, A.; Balaban, T. S.; Holzwarth, A. R.; Schaffner, K. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2861–2863.
- Jesorka, A.; Holzwarth, A. R.; Eichhöfer, A.; Reddy, C. M.; Kinoshita, Y.; Tamiaki, H.; Katterle, M.; Naubron, J.-V.; Balaban, T. S. *Photochem. Photobiol. Sci.* **2012**, *11*, 1069–1080.
- Yagai, S.; Miyatake, T.; Tamiaki, H. *J. Org. Chem.* **2002**, *67*, 49–58.
- Brown, H. C.; Krishnamurthy, S. *J. Am. Chem. Soc.* **1972**, *94*, 7159–7161.
- Other alkyl Grignard reagents have been reacted successfully with methyl pyropheophorbides to give the corresponding 1¹-alkyl-13¹-oxo-chlorins, see: Wang, J.-J.; Han, G.-F.; Shim, Y. K. *J. Photosci.* **2001**, *8*, 75–77.
- (a) Wasielewski, M. R.; Svec, W. A. *J. Org. Chem.* **1980**, *45*, 1969–1974; (b) Smith, K. M.; Goff, D. A.; Simpson, D. J. *J. Am. Chem. Soc.* **1985**, *107*, 4946–4954; (c) Tamiaki, H.; Takeuchi, S.; Tsudzuki, S.; Miyatake, T.; Tanikaga, R. *Tetrahedron* **1998**, *54*, 6699–6718.