Tetrahedron: *Asymmetry* 24 (2013) 677–682

Contents lists available at SciVerse ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

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

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ARTICLE INFO

Article history:
Received 27 March 2013
Accepted 19 April 2013

ABSTRACT

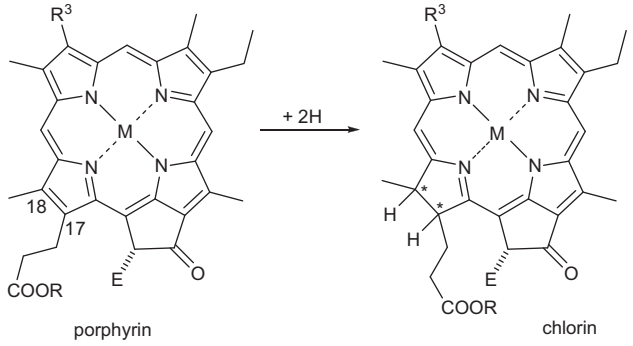
Regioselective reduction of the 13-carbonyl group on the five-membered exo-ring of methyl pyropheophorbide-*a*, one of the chlorophyll-*a* derivatives, with sodium borohydride gave an epimeric mixture of (13^1*R*/*S*)-hydroxy-chlorins. The stereoselectivity was controlled by the steric effect of the (17*S*)-methoxycarbonylethyl group to afford the (13^1*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 13^1-stereogenic center as in the reduced product. All of the anion species (H^-, CH_3^-, and $C_6H_5^-$) favorably attacked the 13^1-carbon atom from the reverse side of the 17-propionate residue, that is, the less sterically crowded face of the 13-C=O plane.

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-$COCH_3$ in a Chl-*a* derivative bearing (17*S*,18*S*)-stereochemistry with an achiral reagent gave a 1:1 3^1-epimeric mixture of the secondary alcohol (Scheme 2b).[4] No chiral induction was observed, partially because the reactive site (C3^1) was distant from the stereogenic centers (C17 and C18) in the molecule: a 1,7/8-induction through six/seven bonds between the C3^1 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 diastereomeric 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–C3^1 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



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*: R^3 = CH=CH_2, E = $COOCH_3$, 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: R^3 = CH_2CH_3 or CH_2OH, E = H, R = CH_3, M = Zn.

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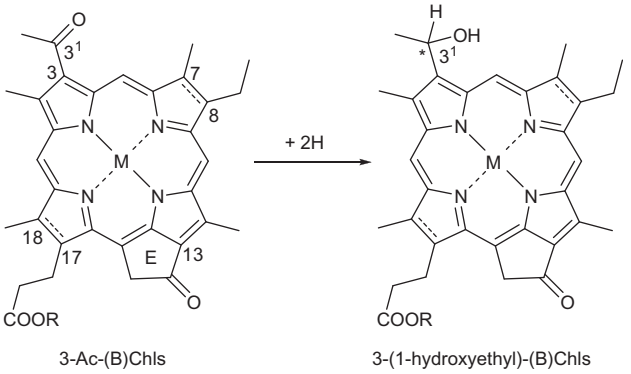
http://dx.doi.org/10.1016/j.tetasy.2013.04.018



Scheme 2. Reduction of 3-acetyl-(B)Chl derivatives to 3-(1-hydroxyethyl) analogues. (a) Stereoselective reduction with a chiral reagent: R = CH_3, M = H_2, C7=C8/C17=C18, C7=C8/C17*S*–C18*S* or C7*R*–C8*R*/C17*S*–C18*S*; (b) non-stereoselective reduction with achiral $NaBH_4$: R = CH_3 or phytyl, M = H_2 or Mg, C7=C8/C17*S*–C18*S* (for a Chl-*a* derivative) or C7*R*–C8*R*/C17*S*–C18*S* (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/C17*S*–C18*S*.

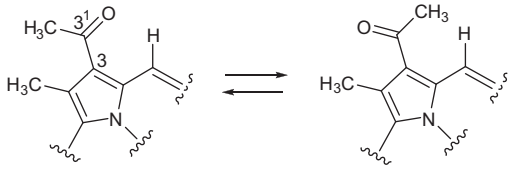
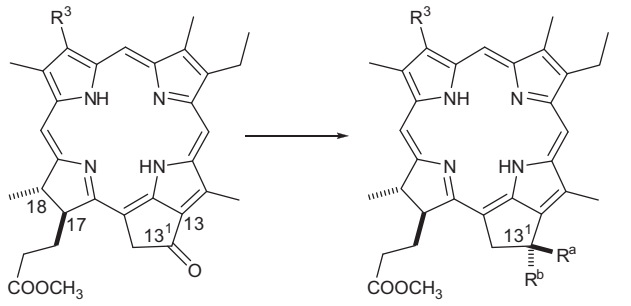


Fig. 1. Conformers (rotamers) around the 3-acetyl group in (B)Chl derivatives.

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 ($NaBH_4$) to give a 13^1-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 $C13^1$ and C17*S*. 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 (17*S*,18*S*)-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 stereochemistry 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.

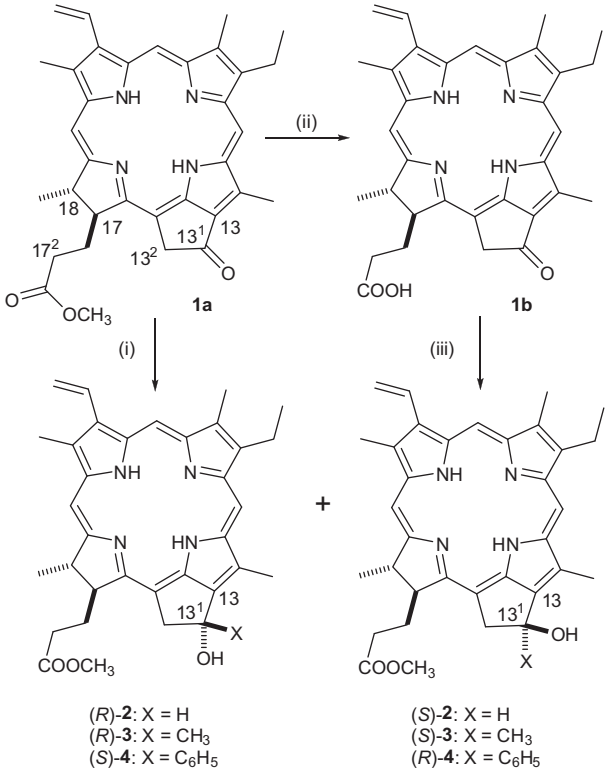


Scheme 3. Reaction of the 13-C=O in (B)Chl derivatives **1** to 13^1-hydroxy-chlorins.

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 17^2-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 $NaBH_4$ occurred regioselectively at the 13-C=O moiety to give methyl 13^1-deoxo-13^1-hydroxy-pyropheophorbide-*a* **2** as the product. The carbinol obtained was a 13^1-epimeric mixture (see the lower drawing of Scheme 4, X = H) and the de values were reported to be 0[14] or 10–20%.[13] Here we re-examined the reduction of **1a** in dichloromethane (CH_2Cl_2) with $NaBH_4$ in methanol (MeOH) at room temperature. The ^{1}H 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-^{1}H NMR including ^{1}H-^{1}H COSY and NOESY in deuterated chloroform ($CDCl_3$) 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 13^2-H^b and removed from the 13^2-H^a, both the 13^2-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) $NaBH_4/CH_2Cl_2$–MeOH or L-selectride/THF, rt; (ii) H_2SO_4/H_2O–THF; (iii) CH_3Li/Et_2O or C_6H_5Li/Bu_2O, TMEDA/THF, −40 or −60 °C to rt and CH_2N_2/Et_2O.

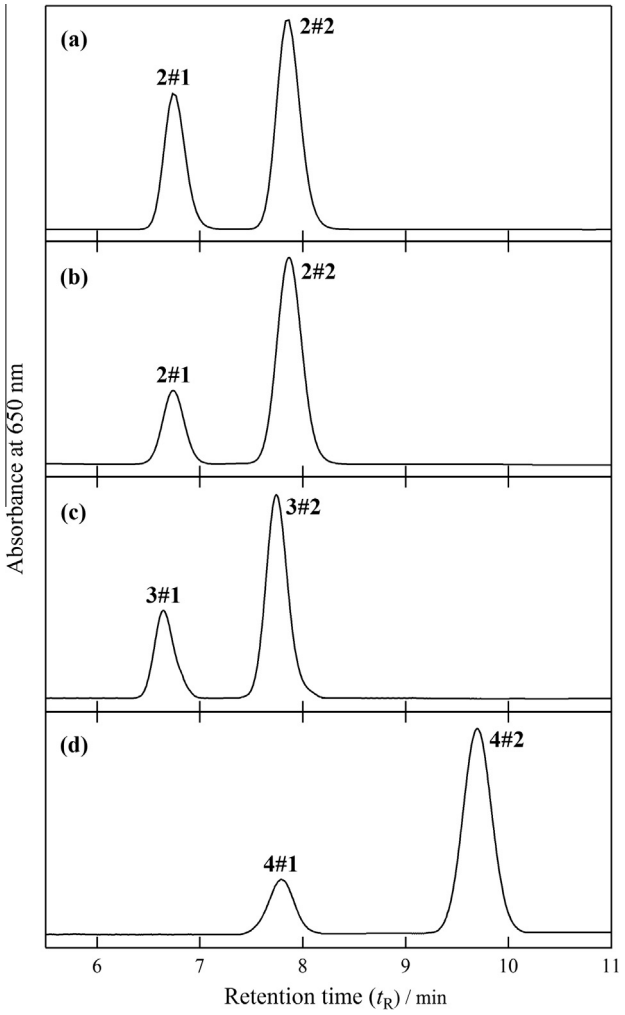


Fig. 2. RP-HPLC of (a) **2#1**/**2** (= **2**(*R*)/(*S*)-**2**, by $NaBH_4$), (b) **2#1**/**2** (= **2**(*R*)/(*S*)-**2**, by L-selectride), (c) **3#1**/**2** (= (*R*/*S*)-**3**), and (d) **4#1**/**2** (= (*R*/*S*)-**4**). Conditions: Cosmosil $5C_{18}$-AR-II (4.6 mm φ × 150 mm), MeOH/H_2O = 95:5 (v/v), and 1.0 mL/min.

assigned from their NOESY spectra (Fig. 3). The NOESY spectrum of **2#1** showed that its 13^1-H was correlated with the 13^2-H^a but not with 13^2-H^b (Fig. 3a). The stereochemistry at the 13^1-position of **2#1** was confirmed to have an (*R*)-configuration: **2#1** = (*R*)-**2**. Based on the NOE-correlation of 13^1-H with 13^2-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 13^1-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

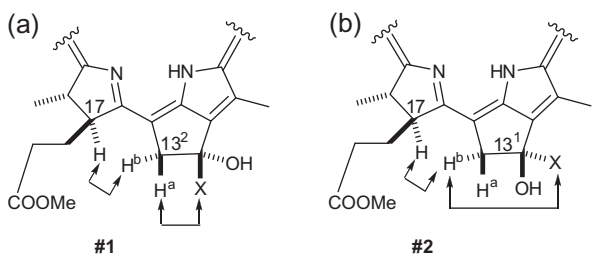


Fig. 3. Specific NOE correlations among the 13^1-X^a/X^b, 13^2-H^a/H^b and 17-H in (a) the first and minor fractions, **2#1** (= (*R*)-**2**) (X = H), **3#1** (= (*R*)-**3**) (X = CH_3), and **4#1** (= (*S*)-**4**) (X = C_6H_5, *ortho*-H); (b) the second and major fractions, **2#2** (= (*S*)-**2**) (X = H), **3#2** (= (*S*)-**3**) (X = CH_3), and **4#2** (= (*R*)-**4**) (X = C_6H_5, *ortho*-H).

of the propionate residue at the 17-position. The two polar substituents, 13^1-OH and 17^2-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 *C*log*P* 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 13^1-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 $NaBH_4$ favorably attacked the partially positively-charged 13^1-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 $NaBH_4$.[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 ^{1}H 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 $NaBH_4$. The de value in the reduction by an L-selectride was 55% and approximately two fold larger than that by $NaBH_4$. 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 $C13^1$-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 CH_3MgI 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 13^2-carbon. The former gave the desired product **3** while the latter afforded a cyclized product through intramolecular Claisen-type reaction with the 17^2-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 (CH_3Li) in diethyl ether (Et_2O) in the presence of *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TMEDA) at −40 °C[9] first produced the lithium carboxylate (17^2-COOLi) and then the 13^1-methylated compound [13-CCH_3(OLi)]. The initially produced salt could not be reacted with the 13^2-anion due to the negative charge on the 17^2-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 ^{1}H NMR. Using the NOE correlations of the 13^1-CH_3 with 13^2-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 *C*log*P* 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 $NaBH_4$. This enhancement could be due to the finding that the methylating reagent (a methyl anion) was bulkier than the reducing reagent (hydride) in $NaBH_4$, 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-postion 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 (C_6H_5Li).

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 ^{1}H 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/CH_3 to C_6H_5 (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: *C*log*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 ^{1}H–^{1}H NOE correlations and HPLC elution order. Their *C*log*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-^{1}H NMR spectra were recorded on a JEOL AL-400 (400 MHz) or ECA-600 (600 MHz) spectrometer, respectively; $CHCl_3$ (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 CH_2Cl_2, *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 μm, 230–400 mesh) or aluminum oxide 90 (activity I, neutral, Merck, 63–200 μm, 70–230 mesh) deactivated by 10% (v/v) H_2O, respectively. RP-HPLC was done with a Shimadzu LC-10ADvp pump and SPD-M10Avp photodiode-array detector: Cosmosil $5C_{18}$-AR-II (4.6 mm φ × 150 mm) as an ODS column and MeOH/H_2O = 95:5 (1.0 mL/min) as an eluent. *C*log*P* values were calculated by a ChemDraw Ultra software (version 8.0.3).

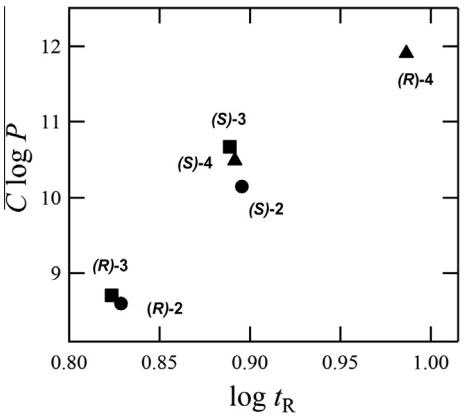


Fig. 4. Correlation between the t_R (min) and *C*log*P* of diastereomers (*R*)- and (*S*)-**2-4**: see Section 4.1 for the RP-HPLC separation conditions.

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 CH_2Cl_2, MeOH, and THF (Nacalai Tesque) were used as the reaction solvents. THF was distilled over calcium hydride just before use. $NaBH_4$ and TMEDA were purchased from Wako Pure Chemical Ind. and Nacalai Tesque, respectively. L-Selectride [LiBH(CHMeEt)$_3$] in THF (ca. 1 M), CH_3Li in Et_2O (1.14 M), and C_6H_5Li in dibutyl ether (Bu_2O) (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 μmol) was dissolved in CH_2Cl_2 (20 mL), to which $NaBH_4$ (80.9 mg, 2.13 mmol) and MeOH (2 mL) were added. The mixture was stirred under N_2 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% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and filtered. After evaporation, the residue was purified by silica gel column chromatography (CH_2Cl_2) to give alcohol **2** (29.5 mg, 53.6 μmol, 57%) as a 1:1.7 mixture of (13^1R)- and (13^1S)-epimers: Vis (CH_2Cl_2) λ_{max} = 652 (relative absorbance, 0.27), 597 (0.03), 546 (0.01), 502 (0.09), 401 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ ($13^1R/S$ = 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, 3^1-CH *trans* to 3-C–H), 6.18 (1H, dd, J = 2, 12 Hz, 3^1-CH *cis* to 3-C–H), 5.41/5.31 (1H, dd, J = 6, 16 Hz, 13^1-CH *cis* to 13-C–H), 4.62/4.76 (1H, d, J = 16 Hz, 13^1-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-CH_2), 3.63 (3H, s, 12-CH_3), 3.57 (3H, s, 2-CH_3), 3.57/3.43 (3H, s, 17^2-$COOCH_3$), 3.42 (3H, s, 7-CH_3), 2.81–2.74, 2.62–2.53, 2.45–2.31, 2.25–2.17 (each 1H, m, 17-CH_2CH_2), 1.85 (3H, d, J = 7 Hz, 18-CH_3), 1.76 (3H, t, J = 8 Hz, 8^1-CH_3), −1.37, −3.22 (each 1H, s, NH × 2); MS (TOF) found: m/z 550.0. Calcd for $C_{34}H_{38}N_4O_3$: 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 (13^1R)-epimer (*R*)-**2** at t_R = 6.7 min and (13^1S)-epimer (*S*)-**2** at 7.9 min. (*R*)-**2**: Vis (CH_2Cl_2) λ_{max} = 653 (relative absorbance, 0.26), 599 (0.03), 544 (0.03), 502 (0.10), 402 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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, 3^1-CH *trans* to 3-C–H), 6.18 (1H, dd, J = 1, 12 Hz, 3^1-CH *cis* to 3-C–H), 5.41 (1H, dd, J = 6, 16 Hz, 13^1-CH *cis* to 13-C–H), 4.65 (2H, q, J = 7.5 Hz, 18-H), 4.63 (1H, d, J = 16 Hz, 13^1-CH *trans* to 13-C–H), 4.47 (1H, d, J = 9 Hz, 17-H), 3.85 (2H, q, J = 7.5 Hz, 8-CH_2), 3.63 (3H, s, 12-CH_3), 3.571 (3H, s, 17^2-$COOCH_3$), 3.565 (3H, s, 2-CH_3), 3.42 (3H, s, 7-CH_3), 2.78–2.71, 2.62–2.54, 2.37–2.31, 2.24–2.18 (each 1H, m, 17-CH_2CH_2), 1.85 (3H, d, J = 7.5 Hz, 18-CH_3), 1.77 (3H, t, J = 7.5 Hz, 8^1-CH_3), −1.34, −3.20 (each 1H, s, NH × 2); MS (TOF) found: m/z 550.6. Calcd for $C_{34}H_{38}N_4O_3$: M^+, 550.3. (*S*)-**2**: Vis (CH_2Cl_2) λ_{max} = 652 (relative absorbance, 0.27), 597 (0.03), 549 (0.01), 502 (0.10), 401 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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, 3^1-CH *trans* to 3-C–H), 6.18 (1H, dd, J = 2, 12 Hz, 3^1-CH *cis* to 3-C–H), 5.30 (1H, dd, J = 6, 16 Hz, 13^1-CH *cis* to 13-C–H), 4.75 (1H, d, J = 16 Hz, 13^1-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-CH_3), 3.57 (3H, s, 2-CH_3), 3.421 (3H, s, 17^2-$COOCH_3$), 3.418 (3H, s, 17-CH_3), 2.81–2.74, 2.61–2.53, 2.45–2.36, 2.24–2.17 (each 1H, m, 17-CH_2CH_2), 1.86 (3H, d, J = 7.5 Hz, 18-CH_3), 1.77 (3H, t, J = 7.5 Hz, 8^1-CH_3), −1.37, −3.32 (each 1H, s, NH × 2); MS (TOF) found: m/z 550.6. Calcd for $C_{34}H_{38}N_4O_3$: 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 μmol) in THF (5 mL) with L-selectride in THF (1 M, 100 μL, 100 μmol) for 30 min gave a 1:3.4 mixture of (*R*)-**2** and (*S*)-**2** (10.9 mg, 19.8 μmol, 20%) and **1a** (42.6 mg, 77.7 μmol) 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 17^2-COOMe to CH_2OH.

4.2.2. Synthesis of methyl 13^1-deoxo-13^1-hydroxy-13^1-methylpyropheophoride-*a* 3

Ketone **1b** (91.2 mg, 171 μmol) was dissolved in THF (40 mL) at −40 °C, to which TMEDA (2 mL, 13.4 mmol) and CH_3Li in Et_2O (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 $CHCl_3$ and the separated organic phase was washed with aq 4% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and filtered. After evaporation, the residue was dissolved in THF (40 mL) and stirred with an excess amount of CH_2N_2 in Et_2O at room temperature for 15 min. All the solvents were distilled in vacuo and the residue was purified by alumina column chromatography (CH_2Cl_2/hexane = 1:3) to give methyl carbinol **3** (32.0 mg, 56.7 μmol, 33%) as a 1:2.3 mixture of (13^1R)- and (13^1S)-epimers: Vis (CH_2Cl_2) λ_{max} = 652 (relative absorbance, 0.28), 596 (0.03), 502 (0.09), 402 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ ($13^1R/S$ = 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, 3^1-CH *trans* to 3-C–H), 6.18 (1H, d, J = 12 Hz, 3^1-CH *cis* to 3-C–H), 5.07/5.03 (1H, d, J = 16 Hz, 13^1-CH *cis* to 13-C–CH_3), 4.84/4.94 (1H, d, J = 16 Hz, 13^1-CH *trans* to 13-C–CH_3), 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-CH_2), 3.62 (3H, s, 12-CH_3), 3.59/3.38 (3H, s, 17^2-$COOCH_3$), 3.57 (3H, s, 2-CH_3), 3.42 (3H, s, 7-CH_3), 2.75–2.71, 2.59–2.48, 2.41–2.33, 2.30–2.23 (each 1H, m, 17-CH_2CH_2), 2.30/2.20 (3H, s, 13^1-CH_3), 1.87 (3H, d, J = 7 Hz, 18-CH_3), 1.78 (3H, t, J = 8 Hz, 8^1-CH_3), −1.47, −3.24 (each 1H, s, NH × 2); MS (TOF) found: m/z 565.8. Calcd for $C_{35}H_{41}N_4O_3$: MH^+, 565.3; HRMS (FAB) found: m/z 565.3176. Calcd for $C_{35}H_{41}N_4O_3$: MH^+, 565.3179.

The above diastereomeric mixture was separated by RP-HPLC to afford (13^1R)-epimer (*R*)-**3** at t_R = 6.7 min and (13^1S)-epimer (*S*)-**3** at 7.7 min. (*R*)-**3**: Vis (CH_2Cl_2) λ_{max} = 653 (relative absorbance, 0.27), 597 (0.03), 502 (0.10), 402 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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, 3^1-CH *trans* to 3-C–H), 6.18 (1H, dd, J = 1.5, 12 Hz, 3^1-CH *cis* to 3-C–H), 5.12 (1H, d, J = 16 Hz, 13^1-CH *cis* to 13-C–CH_3), 4.90 (1H, d, J = 16 Hz, 13^1-CH *trans* to 13-C–CH_3), 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-CH_2), 3.65 (3H, s, 12-CH_3), 3.58 (3H, s, 17^2-$COOCH_3$), 3.57 (3H, s, 2-CH_3), 3.42 (3H, s, 7-CH_3), 2.77–2.71, 2.36–2.22, (each 1H, m, 17-CH_2), 2.63–2.57, 2.24–2.18 (each 1H, m, 17^1-CH_2), 2.42 (3H, s, 13^1-CH_3), 1.84 (3H, d, J = 7.5 Hz, 18-CH_3), 1.76 (3H, t, J = 7.5 Hz, 8^1-CH_3), −3.22 (1H, s, NH) [another NH could not be observed.]; MS (TOF) found: m/z 564.5. Calcd for $C_{35}H_{40}N_4O_3$: M^+, 564.3; HRMS (FAB) found: m/z 565.3180. Calcd for $C_{35}H_{41}N_4O_3$: MH^+, 565.3179. (*S*)-**3**: Vis (CH_2Cl_2) λ_{max} = 652 (relative absorbance, 0.28), 597 (0.03), 503 (0.09), 402 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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, 3^1-CH *trans* to 3-C–H), 6.18 (1H, dd, J = 1.5, 12 Hz, 3^1-CH *cis* to 3-C–H), 5.07 (1H, d, J = 16 Hz, 13^1-CH *cis* to 13-C–CH_3), 4.99 (1H, d, J = 16 Hz, 13^1-CH *trans* to 13-C–CH_3), 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-CH_2), 3.65 (3H, s, 12-CH_3), 3.57 (3H, s, 2-CH_3), 3.42 (3H, s, 7-CH_3), 3.38 (3H, s, 17^2-$COOCH_3$), 2.80–2.72, 2.44–2.37, (each 1H, m, 17-CH_2), 2.59–2.51, 2.23–2.15 (each 1H, m, 17^1-CH_2), 2.32 (3H, s, 13^1-CH_3), 1.87 (3H, d, J = 7.5 Hz, 18-CH_3), 1.76 (3H, t, J = 7.5 Hz, 8^1-CH_3), −1.17, −3.01 (each 1H, s, NH × 2); MS (TOF) found: m/z 564.6. Calcd for $C_{35}H_{40}N_4O_3$: M^+, 564.3; HRMS (FAB) found: m/z 565.3179. Calcd for $C_{35}H_{41}N_4O_3$: MH^+, 565.3179.

4.2.3. Synthesis of methyl 13^1-deoxo-13^1-hydroxy-13^1-phenylpyropheophoride-*a* 4

Similar to the synthesis of **3**, the reaction of **1b** (105 mg, 197 μmol) with C_6H_5Li in Bu_2O (1.9 M, 10 mL, 19 mmol) at −60 °C to room temperature gave phenyl carbinol **4** (8.4 mg, 13.4 μmol, 7%) as a 1:4.5 mixture of (13^1S)- and (13^1R)-epimers: Vis (CH_2Cl_2) λ_{max} = 653 (relative absorbance, 0.29), 598 (0.03), 502 (0.10), 402 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ ($13^1S/R$ = 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 13^1-Ph), 7.41/7.38 (2H, t, J = 7.5 Hz, *m*-H of 13^1-Ph), 7.38/7.32 (1H, t, J = 7.5 Hz, *p*-H of 13^1-Ph), 6.37 (1H, d, J = 18 Hz, 3^1-CH *trans* to 3-C–H), 6.19 (1H, d, J = 12 Hz, 3^1-CH *cis* to 3-C–H), 5.31/5.24 (1H, d, J = 16 Hz, 13^1-CH *cis* to 13-C–Ph), 5.18/5.37 (1H, d, J = 16 Hz, 13^1-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-CH_2), 3.58 (3H, s, 2-CH_3), 3.55/3.31 (3H, s, 17^2-$COOCH_3$), 3.42 (3H, s, 7-CH_3), 3.39 (3H, s, 12-CH_3), 2.79–2.74, 2.57–2.51, 2.48–2.43, 2.20–2.15 (each 1H, m, 17-CH_2CH_2), 1.86 (3H, d, J = 7 Hz, 18-CH_3), 1.76 (3H, t, J = 8 Hz, 8^1-CH_3), −1.47, −3.24 (each 1H, s, NH × 2); MS (TOF) found: m/z 626.7. Calcd for $C_{40}H_{42}N_4O_3$: M^+, 626.3; HRMS (FAB) found: m/z 627.3315. Calcd for $C_{40}H_{43}N_4O_3$: MH^+, 627.3335.

The above diastereomeric mixture was separated by RP-HPLC to afford (13^1S)-epimer (*S*)-**4** at t_R = 7.8 min and (13^1R)-epimer (*R*)-**4** at 9.7 min. (*S*)-**4**: Vis (CH_2Cl_2) λ_{max} = 653 (relative absorbance, 0.29), 598 (0.03), 502 (0.09), 402 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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 13^1-Ph), 7.46 (2H, t, J = 7.5 Hz, *m*-H of 13^1-Ph), 7.38 (1H, t, J = 7.5 Hz, *p*-H of 13^1-Ph), 6.35 (1H, dd, J = 1.5, 18 Hz, 3^1-CH *trans* to 3-C–H), 6.19 (1H, dd, J = 1.5, 12 Hz, 3^1-CH *cis* to 3-C–H), 5.34 (1H, d, J = 16 Hz, 13^1-CH *cis* to 13-C–Ph), 5.18 (1H, d, J = 16 Hz, 13^1-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-CH_2), 3.57 (3H, s, 2-CH_3), 3.55 (3H, s, 17^2-$COOCH_3$), 3.42 (3H, s, 7-CH_3), 3.34 (3H, s, 12-CH_3), 2.73–2.71, 2.25–2.20, (each 1H, m, 17-CH_2), 2.62–2.54, 2.31–2.26 (each 1H, m, 17^1-CH_2), 1.85 (3H, d, J = 7.5 Hz, 18-CH_3), 1.75 (3H, t, J = 7.5 Hz, 8^1-CH_3), −1.25, −3.22 (each 1H, s, NH × 2); MS (TOF) found: m/z 625.7. Calcd for $C_{40}H_{42}N_4O_3$: M^+, 626.3; HRMS (FAB) found: m/z 626.3249. Calcd for $C_{40}H_{42}N_4O_3$: M^+, 626.3257. (*R*)-**4**: Vis (CH_2Cl_2) λ_{max} = 653 (relative absorbance, 0.29), 598 (0.03), 502 (0.09), 402 nm (1.00); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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 13^1-Ph), 7.38 (2H, t, J = 7.5 Hz, *m*-H of 13^1-Ph), 7.32 (1H, t, J = 7.5 Hz, *p*-H of 13^1-Ph), 6.37 (1H, d, J = 18 Hz, 3^1-CH *trans* to 3-C–H), 6.19 (1H, d, J = 12 Hz, 3^1-CH *cis* to 3-C–H), 5.37 (1H, d, J = 16 Hz, 13^1-CH *trans* to 13-C–Ph), 5.24 (1H, d, J = 16 Hz, 13^1-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-CH_2), 3.58 (3H, s, 2-CH_3), 3.42 (3H, s, 7-CH_3), 3.39 (3H, s, 12-CH_3), 3.30 (3H, s, 17^2-$COOCH_3$), 2.80–2.72, 2.48–2.42, (each 1H, m, 17-CH_2), 2.54–2.50, 2.23–2.17 (each 1H, m, 17^1-CH_2), 1.86 (3H, d, J = 7.5 Hz, 18-CH_3), 1.76 (3H, t, J = 7.5 Hz, 8^1-CH_3), −1.27, −3.17 (each 1H, s, NH × 2); MS (TOF) found: m/z 626.1. Calcd for $C_{40}H_{42}N_4O_3$: M^+, 626.3; HRMS (FAB) found: m/z 626.3261. Calcd for $C_{40}H_{42}N_4O_3$: M^+, 626.3257.

Acknowledgments

This work was partially supported by a Grant-in-Aid for Scientific Research (A) (No. 22245030) from the Japan Society for the Promotion of Science (JSPS).

References

1. (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-Kurdziel, B. *Photosynthetica* **2012**, *50*, 529–540; (d) Moser, J.; Lange, C.; Krausze, J.; Rebelein, J.; Schubert, W.-D.; Ribbe, M. W.; Heinz, D. W.; Jahn, D. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 2094–2098.
2. (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.
3. Tamiaki, H.; Kouraba, M.; Takeda, K.; Kondo, S.; Tanikaga, R. *Tetrahedron: Asymmetry* **1998**, *9*, 2101–2111.
4. (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.
5. (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.
6. (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.
7. Tamiaki, H.; Shibata, R.; Mizoguchi, T. *Photochem. Photobiol.* **2007**, *83*, 152–162.
8. Tamiaki, H.; Miyatake, T.; Tanikaga, R. *Tetrahedron Lett.* **1997**, *38*, 267–270.
9. Yagai, S.; Miyatake, T.; Shimono, Y.; Tamiaki, H. *Photochem. Photobiol.* **2001**, *73*, 153–163.
10. Tamiaki, H.; Kunieda, M. *Handb. Porphyr. Sci.* **2011**, *11*, 223–290.
11. Wolf, H.; Scheer, H. *Justus Liebigs Ann. Chem.* **1971**, *745*, 87–98.
12. 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.
13. (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.
14. Jesorka, A.; Balaban, T. S.; Holzwarth, A. R.; Schaffner, K. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2861–2863.
15. 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.
16. Yagai, S.; Miyatake, T.; Tamiaki, H. *J. Org. Chem.* **2002**, *67*, 49–58.
17. Brown, H. C.; Krishnamurthy, S. *J. Am. Chem. Soc.* **1972**, *94*, 7159–7161.
18. Other alkyl Grignard reagents have been reacted successfully with methyl pyropheophorbides to give the corresponding 13^1-alkyl-13^1-oxo-chlorins, see: Wang, J.-J.; Han, G.-F.; Shim, Y. K. *J. Photosci.* **2001**, *8*, 75–77.
19. (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.