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Asymmetric synthesis of methyl bacteriopheophorbide-*d* and analogues by stereoselective reduction of the 3-acetyl to the 3-(1-hydroxyethyl) group

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Abstract

Asymmetric borane-reduction of 3-acetyl-13^1-oxo-tetrapyrroles (=bacteriochlorin, chlorin and porphyrin) in the presence of a chiral source gave selectively chiral 3-(1-hydroxyethyl)-13^1-oxo-tetrapyrroles. Oxazaborolidines were effective as chiral auxiliaries. Reduction with (*S*)-oxazaborolidines led to the (3^1*S*)-alcohol as the major product, whose stereoselectivity was the opposite of that in the same asymmetric reduction of usual prochiral ketones ArCOR. The asymmetric reduction of the 3-acetyl group in methyl bacteriopyropheophorbide-*a* and the 7,8-oxidation afforded 3^1-epimeric methyl (3^1*S*)-bacteriopheophorbide-*d* (89% de). © 1998 Published by Elsevier Science Ltd. All rights reserved.

1. Introduction

Naturally occurring bacteriochlorophyll-*d* (=BChl-*d*, see Fig. 1), one of the major pigments in light-harvesting antennae of green photosynthetic bacteria, is a 3^1-epimeric mixture.[1] Several reports[2] have proposed that the 3^1-configuration should control the supramolecular structures of the photosynthetic antennae. We and Holzwarth et al.[3,4] showed that model systems including self-aggregates of Zn-**1a** (see Fig. 1) in non-polar organic solvents built up a similar structure with the in vivo antennae, and the 3^1-diastereoselective controls operated in the models. 3^1-Epimerically pure BChl-*d* and Zn-**1a** were prepared by HPLC separation of the epimeric mixture.[3,4] It has been difficult to date to get a large amount of the 3^1-epimer. Here we report on the asymmetric synthesis of methyl bacteriopheophorbide-*d* **1a** by stereoselective borane-reduction of the acetyl group with chiral oxazaborolidines.

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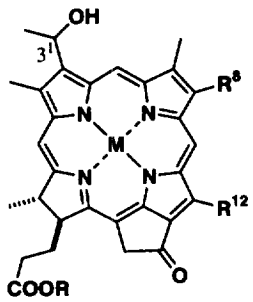


Fig. 1. Bacteriochlorophyll-*d* (BChl-*d*): M=Mg; R^8=Et, Pr, *iso*-Bu, *neo*-Pn; R^{12}=Me, Et; R=farnesyl. Zinc methyl bacteriopheophorbide-*d* (Zn-**1a**): M=Zn; R^8=Et; R^{12}=R=Me

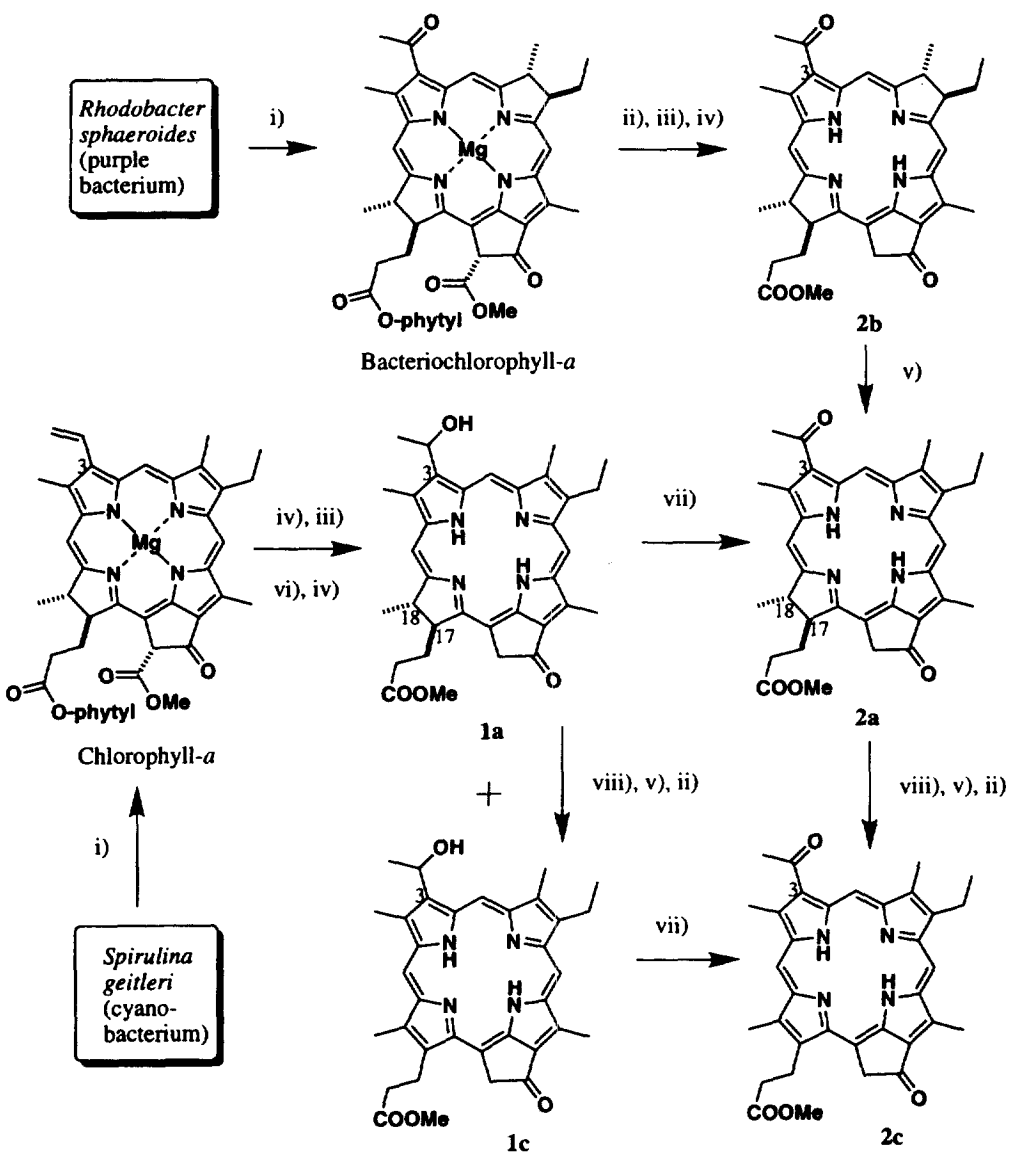
2. Results and discussion

2.1. Synthesis of 3-acetyl-tetrapyrroles 2

According to the reported procedure,[3,5] methyl bacteriopheophorbide-*d* **1a** was synthesized from chlorophyll-*a* which was extracted from a cyanobacterium, *Spirulina geitleri* (see Scheme 1). Oxidation of the 1-hydroxyethyl group at the 3-position of **1a** gave 3-acetyl-chlorin **2a**. During hydration of the 3-vinyl group of methyl pyropheophorbide-*a*, 3-(1-hydroxyethyl)-porphyrin **1c** was produced as well as the desired **1a**.[6] The 3-CH(OH)Me of **1c** was oxidized to give 3-acetyl-porphyrin **2c**. Porphyrin **2c** was also afforded by zinc-metallation, 17,18-oxidation and demetallation[6] of chlorin **2a**. Methyl bacteriopyropheophorbide-*a* **2b** was synthesized from bacteriochlorophyll-*a* which was extracted from a purple bacterium, *Rhodobacter sphaeroides* (see Scheme 1). All the synthetic tetrapyrroles were characterized by ^{1}H-NMR, visible and mass spectra.

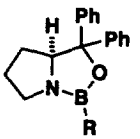
2.2. Asymmetric reduction of 2 to 1

Recently, Salunkhe and Burkhardt[7] reported that prochiral ketones were reduced highly enantioselectively using borane-*N*,*N*-diethylaniline as a reductant and oxazaborolidines as a chiral catalyst, which was a modification of the procedures developed by Corey et al.[8] This prompted us to investigate asymmetric synthesis of methyl bacteriopheophorbide-*d* **1a**. Following the procedure,[7] 4 mM of a toluene solution of 3-acetylchlorin **2a** was treated with 100 mol% $BH_3 \cdot PhNEt_2$ and 5 mol% of (*S*)-methyloxazaborolidine (*S*)-**3a** (see Fig. 2) to give no product. The reason for this lack of success was the lower than 1 M concentration of the solution of ketones reported in the literature,[7] and could also be due to small amounts of impurities, including moisture, originating in toluene as the solvent. To overcome this problem, larger amounts of the reductant and the chiral source were used so that ketone **2a** was completely consumed within 20 min at 30°C and **1a** was afforded in 47% yield (entry 1 in Table 1). The low yield is due to the reduction of the 13^1-keto group. Use of borane–tetrahydrofuran (=THF) as the borane source suppressed the undesired reduction and induced the predominant formation of the desired **1a** (entries 2 and 3). The diastereoselectivity was about 3:1, as determined from chiral-phase HPLC shown in Fig. 3A. The absolute configuration of the produced alcohol **1a** was unambiguously determined in comparison with the reported ^{1}H NMR data of the authentic sample.[3,5] The major diastereomer in the asymmetric reduction was (3^1*S*)-**1a**. The stereoselectivity was the opposite of that reported so far;[9] a typical reduction of acetophenone with $BH_3 \cdot$(*S*)-**3a** gave (*R*)-1-phenylethanol. This reversed selectivity was attributed to the structure of chlorin as a prochiral ketone rather than to a large amount of $BH_3 \cdot$(*S*)-**3a** in the reaction mixture.



Scheme 1. Synthesis of 3-acetyl-tetrapyrroles **2**: (i) extraction; (ii) dil. HCl; (iii) collidine, reflux; (iv) H_2SO_4/MeOH; (v) DDQ/acetone; (vi) HBr/AcOH; (vii) Pr_4NRuO_4–Me(O)N$(CH_2CH_2)_2O$/CH_2Cl_2; (viii) $Zn(OAc)_2$/MeOH–CH_2Cl_2

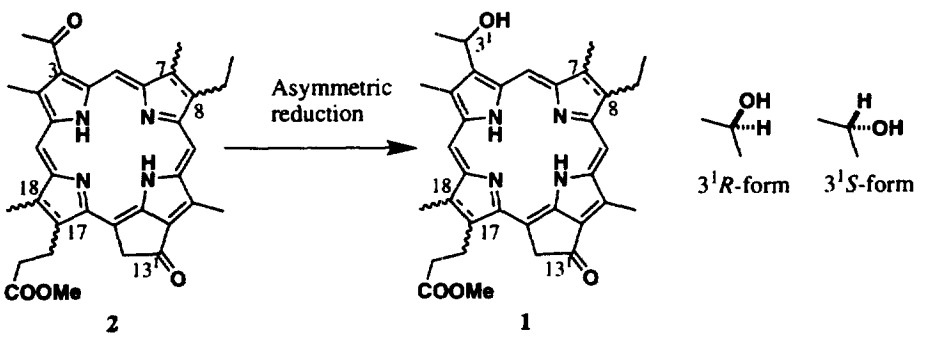
Asymmetric reduction of other 3-acetyl-tetrapyrroles was examined. When 3-acetyl-13^1-oxo-bacteriochlorin **2b** (=7,8-reduced form of **2a**) was reduced under similar conditions, the corresponding alcohol **1b** was site-selectively and rapidly produced in a high yield (93%, entry 4). Treatment of bacteriochlorin **1b** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ)[10] gave the corresponding chlorin **1a** quantitatively. The mild DDQ-oxidation induced no epimerization of the 3^1-position. The HPLC analysis of **1a** derived from **1b** showed that high (*S*)-diastereoselective reduction was achieved in **2b**→(3^1*S*)- and (3^1*R*)-**1b** (94:6). Although achiral 3-acetyl-13^1-oxo-porphyrin **2c** (=17,18-oxidized form of **2a**) was reduced over a prolonged period, the consumption of **2c** was 39% and the yield of **1c** was 30% (entry 5). The enantioselectivity was determined to be 26% ee from the chiral HPLC analysis



(3¹*S*)-**3a**: R = Me
(3¹*S*)-**3b**: R = H

Fig. 2. Oxazaborolidines **3**

Table 1
Asymmetric reduction of **2** to **1**[a]



2 **1**

a (chlorin): C^7=C^8, C^{17}H–C^{18}H (17*S*,18*S*)
b (bacteriochlorin): C^7H–C^8H (7*R*,8*R*), C^{17}H–C^{18}H (17*S*,18*S*)
c (porphyrin): C^7=C^8, C^{17}=C^{18}

Entry	Chiral auxiliary	Reductant	Solvent	Reaction time / h	Prochiral ketone	Conversion / %	Yield / %	De / %	Major alcohol
1	(*S*)-**3a**	BH_3·PhNEt$_2$	PhMe	0.5	**2a**	100	47	48	(*S*)-**1a**
2	(*S*)-**3a**	BH_3·THF	PhMe	0.5	**2a**	90	84	43	(*S*)-**1a**
3	(*S*)-**3a**	BH_3·THF	THF	0.5	**2a**	90	84	46	(*S*)-**1a**
4	(*S*)-**3a**	BH_3·THF	THF	0.3	**2b**	100	93	88	(*S*)-**1b**
5	(*S*)-**3a**	BH_3·THF	THF	3	**2c**	39	30	26[b]	(*S*)-**1c**
6	(*S*)-**3b**	BH_3·Me_2S	PhMe-THF	5	**2a**	100	93	48	(*S*)-**1a**

[a] **2** : **3a** : reductant = 1 : 3.7 : 5.6 at 30 °C; **2** : **3b** : reductant = 1 : 13 : 23 in PhMe-THF (1:1) at 45 °C. [b] Ee / %.

as shown in Fig. 3B. According to the reported procedure (zinc-metallation, 17,18-oxidation by DDQ and demetallation),[6] chiral **1a** was transformed to the corresponding **1c** without epimerization of the 3¹-position. The first eluted band in chiral HPLC of **1c** was assigned to the (3¹*S*)-enantiomer and the second to the (3¹*R*)-enantiomer. The (*S*)-selectivity was also observed in the enantiomeric reduction. As the tetrapyrrole chromophores were reduced (**2c**→**2a**→**2b**), the reactivity of the 3-acetyl group increased in the asymmetric reduction as well as stereoselectivity.

The present stereoselectivity was opposite to that proposed in asymmetric reduction of another 3-acetylporphyrin:[11] 78% ee of the (*R*)-alcohol by BH_3·(*S*)-**3a**. Under the same conditions as described

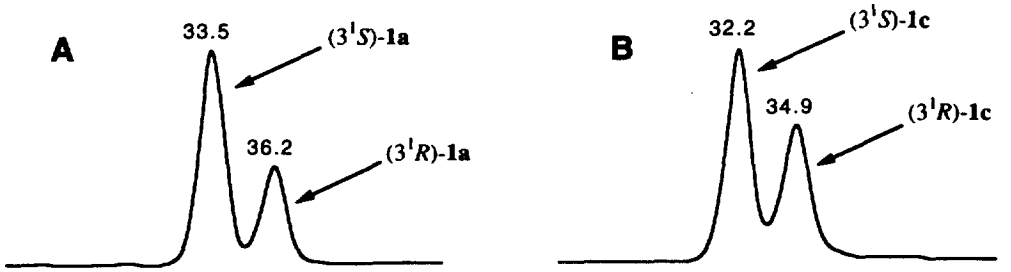


Fig. 3. High performance liquid chromatograms of $(3^1S)/(3^1R)$-**1a** (**A**, separation ratio (R_s)=1.2) and $(3^1S)/(3^1R)$-**1c** (**B**, (R_s)=1.1) by a chiral-phase column (SUMICHIRAL OA-4400, 4.6 ϕ×250 mm, hexane/CH_2ClCH_2Cl/MeOH=76/24/0.3, 1.0 ml/min)

in the literature (0.9 equiv. $BH_3 \cdot Me_2S$ and 0.4 equiv. (*S*)-**3a** in CH_2Cl_2),[11] reduction of **2a** also gave (3^1S)-**1a** as the major product. Therefore, asymmetric reduction of 3-acetyl-13^1-oxo-tetrapyrroles **2** in the present work induced a reversed stereoselectivity compared with the enantiomeric BH_3-reduction of the usual prochiral ketone by chiral oxazaborolidine catalyst.[8,9]

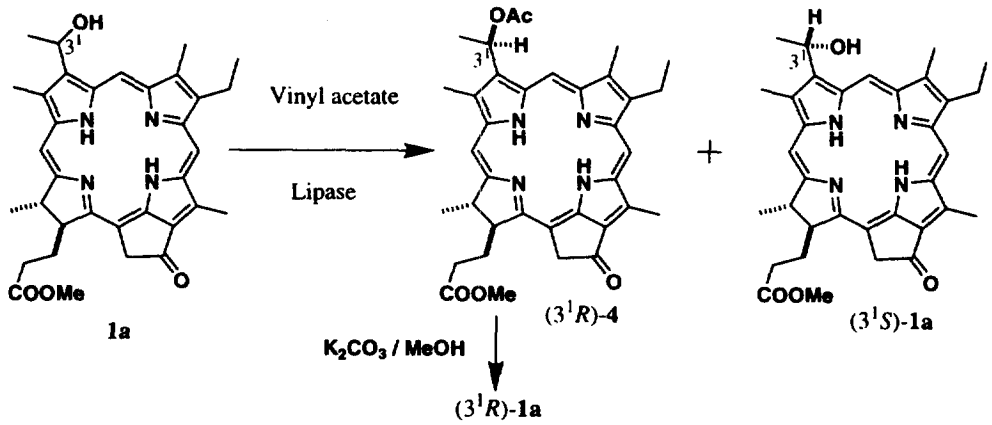
Chiral **3b** (=demethylated form of **3a**) was examined for the synthesis of chiral **1**. According to the procedure reported by Prasad and Joshi,[12] in situ generated $BH_3 \cdot$(*S*)-**3b** was used for the reduction of **2a** (entry 6). The result was almost the same as that by **3a** and (*S*)-selectivity was also observed. As a result, **3b** was also effective for the stereoselective reduction of **2** to **1**.

Asymmetric reductions of **2a** to **1a** by other chiral reductants were investigated. Reaction of **2a** with (−)-chlorodiisopinocamphenylboran originally prepared by Brown and his colleagues[13] could not be observed. According to the procedure reported by Noyori et al.,[14] reduction of **2a** with LiAlH(OEt)((*R*)-1,1′-bi-2-naphthoxy) gave (3^1R)-**1a** in a low yield (5%) and a low diastereoselectivity (11% de). Reduction of **2a** with *N*,*N*′-bis[2-(mesitoyl)-3-oxobutylidene]-(1*S*,2*S*)-1,2-diphenylethylenediaminato cobalt(II)–$NaBH_2$(OEt)(OCH_2-2-furyl) reported recently by Sugi, Mukaiyama and their colleagues[15] gave (3^1S)-**1a** in low yield (18%) and a relatively high diastereoselectivity (54% de). The latter two reductions of **2a** gave **1a** in lower yields than reductions by oxazaborolidine **3**. The configurations of major epimer **1a** produced by the two reductions were consistent with the stereoisomers expected from the proposed mechanisms.

2.3. *Kinetic resolution of* **1a** *by lipase*

Lipase catalyzed transesterification of racemic alcohol or the hydrolysis of a racemic ester have been useful tools for the construction of a stereogenic center. Lipase catalyzed transesterification of 1-arylethanols such as 1-phenylethanol gave especially good results.[16] The structural similarity of **1a** with 1-arylethanol encouraged us to study the kinetic resolution of **1a** catalyzed by lipases and esterase. A screening of lipase (Table 2) showed that two lipase, Amano PS and Toyobo, catalyzed the transesterification of **1a** to give the corresponding acetate **4** in toluene with vinyl acetate as an acyl donor (entries 1 and 2), although the reactions were very slow compared to those of other 1-arylethanols. Other lipases and esterase (entries 3–6) gave no product after 3 days. Diastereomeric excess and configuration of the 3^1-position of residual alcohol **1a** were determined by HPLC and ^{1}H NMR. The de of **4** was also determined after conversion into **1a** by methanolysis in the presence of K_2CO_3. Enantioselectivity on the 3^1-position was the same as in the case of 1-arylethanol to give the (*R*)-acetate. E-values[17] for transesterification of **1a** were only 2.9 and 2.0 as catalyzed by Toyobo lipase and Amano PS, respectively. These low values arose because of the large size of the chlorin moiety and/or the steric hindrance around

Table 2
Kinetic resolution of **1a** by lipase catalyzed transesterification[a]



Entry	Lipase Esterase	Conversion / %	De of **4** / % (configuration)	De of **1a** / % (configuration)	E-value
1	Toyobo Lipase[b]	24	44 (3^1R)	14 (3^1S)	2.9
2	Amano PS[b]	42	26 (3^1R)	19 (3^1S)	2.0
3	CCL[c]	0	—	—	—
4	PPL[d]	0	—	—	—
5	CAL[e]	0	—	—	—
6	PLE[f]	0	—	—	—

[a] **1a**, 100 mg; $AcOCH{=}CH_2$, 20 ml; lipase, 400 mg; molecular sieves 3A, 8.0 g; toluene, 100 ml; 40 °C; 110 h.

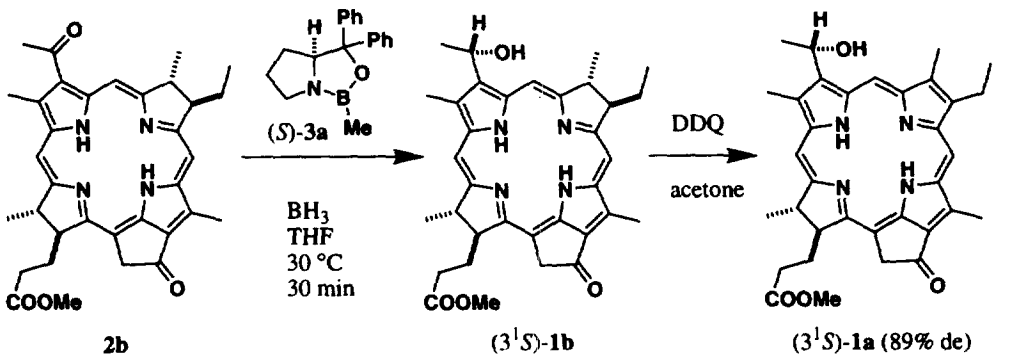
[b] From *Pseudomonas* sp. [c] From *Candida cylindracea* (Sigma). [d] From porcine pancrea (Sigma). [e] From *Candida antarctica*. [f] From pig liver.

the 3-(1-hydroxyethyl) group to bind into the active site of the enzyme,[18] which also caused the low catalytic activity.

2.4. *Stereoselective synthesis of methyl bacteriopheophorbide-*d ***1a***

Methyl bacteriopyropheo- phorbide-*a* **2b** easily prepared from purple photosynthetic bacteria, was regio- and stereoselectively reduced by borane-(*S*)-oxazaborolidine **3a** (or **3b**) in THF and successively oxidized by DDQ in acetone to give efficiently methyl (3^1S)-bacteriopheophorbide-*d* **1a** in 89% de (see Scheme 2). This route is superior to the direct reduction of **2a** to **1a** (less than 50% de). Use of (*R*)-oxazaborolidine **3a** (or **3b**) as a chiral auxiliary led to the predominant formation of (3^1R)-**1a**. The present stereoselective synthesis of **1a** would allow preparation of a large amount of 3^1-epimerically pure BChl-*d*.

It must be noted that asymmetric reduction of 3-acetyl-13^1-oxo-tetrapyroles **2** by BH_3·(*R*)/(*S*)-**3** gave (3^1R)/(3^1S)-**1** as the major epimer, respectively, while ArCOR (Ar=aromatic, R=alkyl groups) was



Scheme 2. Stereoselective synthesis of methyl (3¹*S*)-bacteriopheophorbide-*d* (3¹*S*)-**1a**

usually reduced to (*S*)/(*R*)-ArCH(OH)R by the same BH_3·(*R*)/(*S*)-**3**. This reversed stereoselectivity is due to large multi-functionalized tetrapyrrole chromophores; steric repulsion and/or electronic interaction between the tetrapyrroles of **2** and some substituents of BH_3·**3** might occur in the reaction intermediate.

3. Experimental

3.1. General

All of the equipment was described in a previous report.[19] Chemical shifts (δ) of ^{1}H-NMR spectra are expressed in parts per million relative to $CHCl_3$ as an internal reference. (*S*)-5,5-Diphenyl-2-methyl-3,4-propano-1,3,2-oxazaborolidine (*S*)-**3a**, (*R*)-/(*S*)-2-[hydroxy(diphenyl)methyl]-1-methylpyrrodine (diphenyl prolinol), and *N*,*N*′-bis[2-(mesitoyl)-3-oxobutylidene]-(1*S*,2*S*)-1,2-diphenylethylenediaminato cobalt(II) were purchased from Tokyo Chemical Industry and (−)-chlorodiisopinocamphenylboran ((−)-DIP-Chloride™) was purchased from Aldrich. Flash column chromatography (FCC) was performed with silica gel (Merck, Kieselgel 60, 9385). HPLC for separation of 3¹-epimers (3¹*S*)/(3¹*R*)-**1a** and (3¹*S*)/(3¹*R*)-**1c** was performed with a chiral column (SUMICHIRAL OA-4400, 4.6 ϕ×250 mm, Sumika Chemical Analysis Service, hexane/CH_2ClCH_2Cl/MeOH=76/24/0.3, 1.0 ml/min). All synthetic procedures had to be done in the dark.

3.2. Oxidation of 1-hydroxyethyl to acetyl group[20]

4-Methylmorpholine-*N*-oxide (24 mg) and tetrapropylammonium perruthenate (10 mg) were added to a dry CH_2Cl_2 (20 ml) solution of 3-(1-hydroxyethyl)tetrapyrrole **1** (0.1 mmol) and stirred at room temperature under N_2 for 2 h. The reaction mixture was poured into H_2O, extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by FCC and recrystallization to give the corresponding 3-acetyl-tetrapyrrole **2**.

3.3. 7,8-Oxidation of a bacteriochlorin to a chlorin[10]

A dry acetone solution (6 ml) of DDQ (13.6 mg) was added to a dry acetone solution (20 ml) of 13¹-oxo-bacteriochlorin (18 μmol) and then stirred at room temperature under N_2. The reaction mixture was poured into ice–water and extracted with CH_2Cl_2, washed with H_2O, aq. sat. $NaHCO_3$ and brine, and

dried over Na_2SO_4. After evaporation, the residue was purified by FCC and recrystallized to give the corresponding 13^1-oxo-chlorin.

3.4. 17,18-Oxidation of a chlorin to a porphyrin[6]

Zinc metallation of a 13^1-oxo-chlorin, 17,18-oxidation by 1 equivalent of DDQ in dry acetone for a few minutes and demetallation by treatment with concentrated HCl gave the corresponding 13^1-oxo-porphyrin.

3.5. Asymmetric reduction

(*S*)-Methyloxazaborolidine (*S*)-**3a** (18.0 mg, 64.9 μmol, 3.7 equiv.) and BH_3/THF (1 M solution, 10.0 μl, 100 μmol, 5.6 equiv.) was dissolved in 2 ml THF. The solution was stirred at 30°C under N_2. 3-Acetyl-13^1-oxo-tetrapyrrole **2** (17.7 μmol) in 2 ml of THF was placed in a dropping funnel, and slowly added to the solution over 15 min. After the addition, the reaction mixture was monitored by visible spectra. When the decrease of the peaks in **2** had stopped with the appearance of blue-shifted peaks of **1**, the reaction mixture was carefully quenched with 5 ml of MeOH, followed by addition of aq. 2% HCl and was stirred for 15–20 min. The aqueous phase was extracted with several portions of CH_2Cl_2 and the combined CH_2Cl_2 phases were washed with aq. 4% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by FCC with Et_2O/CH_2Cl_2 and recrystallization from CH_2Cl_2/hexane to give (3^1S)-alcohol **1** as the major epimer.

To a solution of $BH_3 \cdot Me_2S$/toluene (0.5 M solution, 1.3 ml, 650 μmol, 36.7 equiv.) a solution of (*S*)-diphenyl prolinol (63.5 mg, 237 μmol, 13.4 equiv.) in THF (2 ml) was added, and the reaction mixture was stirred at 45°C for 16 h under N_2. 3-Acetyl-13^1-oxo-tetrapyrrole **2** (17.7 μmol) in 2 ml of THF was added slowly. After completion of the reaction monitored by the visible spectra, similar work-up as described above gave (*S*)-rich alcohol **1**.

3.6. Kinetic resolution

To a suspension of vinyl acetate (20 ml), 3 Å molecular sieves (8.0 g) and lipase (400 mg) in toluene (100 ml) was added **1a** (100 mg) and the mixture was stirred at 40°C for 110 h under N_2. After filtration, the residue was washed with CH_2Cl_2. The filtrate was evaporated *in vacuo* and purified by FCC (Et_2O/CH_2Cl_2) to give alcohol **1a** and the acetate **4**. Solvolysis of **4** by 0.05 M K_2CO_3 in MeOH at room temperature gave **1a** after purification by FCC.

3.7. Methyl bacteriopheophorbide-d *1a*

Following the reported procedure,[3,5] hydration of the 3-vinyl group of methyl pyropheophorbide-*a* gave **1a**.[3] Alternatively, 7,8-oxidation of methyl 3-deacetyl-3-(1-hydroxyethyl)-bacteriopyropheophorbide-*a* **1b** for <1 min afforded **1a** (>90% yield) after purification by FCC (10–16% Et_2O/CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane.

3.8. Methyl 3-deacetyl-3-(1-hydroxyethyl)bacteriopyropheophorbide-a *1b*

To a CH_2Cl_2 (30 ml) solution of **2b** (50 mg) was added a MeOH (5 ml) solution of $NaBH_4$ (30 mg) and the mixture was stirred at 0°C under N_2 for 20 min. The reaction mixture was poured into H_2O, extracted

with CH_2Cl_2, washed with aq. 4% $NaHCO_3$ and brine, and dried over Na_2SO_4. After evaporation, the residue was purified by FCC (10–15% Et_2O/CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane to give **1b** (46 mg; 92% yield, $(3^1R)/(3^1S)$=1/1); black solids; VIS (CH_2Cl_2) λ_{max}=712 (0.40), 651 (0.12), 599 (0.044), 512 (0.30), 482 (0.067), 452 (0.024), 379 (0.53), 352 (1.00); ^1H NMR ($CDCl_3$) δ=8.51 (1H, s, 5-H), 8.20 (1H, s, 10-H), 7.99 (1H, s, 20-H), 6.16 (1H, q, *J*=6 Hz, 3^1-H), 4.94, 4.76 (1H+1H, d, *J*=20 Hz, 13^2-H_2), 4.11 (2H, br-q, *J*=7 Hz, 17-, 18-H), 3.94 (1H, dt, *J*=8, 2 Hz, 17-H), 3.87 (1H, dt, *J*=8, 4 Hz, 8-H), 3.61 (3H, s, $COOCH_3$), 3.34 (3H, s, 12-CH_3), 3.19 (3H, s, 2-CH_3), 2.40–2.56, 2.12–2.33, 1.96–2.06 (2H+3H+1H, m, 8-CH_2, 17-CH_2CH_2), 2.04 (3H, d, *J*=6 Hz, 3^1-CH_3), 1.75/1.74, 1.64 (3H+3H, d, *J*=7 Hz, 7-, 18-CH_3), 1.10 (3H, t, *J*=7 Hz, 8^1-CH_3), 1.35, −0.19 (1H+1H, s, NH). MS (FAB) found: *m/z* 568. Calcd for $C_{34}H_{40}N_4O_4$: M^+, 568.

3.9. *Methyl protobacteriopheophorbide*-d **1c**

Using the procedure by Tamiaki and his colleagues,[6] hydration of the 3-vinyl group of methyl pyropheophorbide-*a* and concomitantly 17,18-oxidation under the acidic conditions gave **1c**.[6] Alternatively, 17,18-oxidation of methyl bacteriopheophorbide-*d* **1a** afforded **1c** (67% yield) after purification by FCC (15% Et_2O/CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane.

3.10. *Methyl 3-acetyl-3-devinyl-pyropheophorbide*-a **2a**

Oxidation of the 3-(1-hydroxyethyl) group of **1a** gave **2a**.[20] Alternatively, 7,8-oxidation of **2b** for 20 min afforded **2a** (79% yield) after purification by FCC (5–8% Et_2O/CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane.

3.11. *Methyl bacteriopyropheophorbide*-a **2b**

After complete cultivation of *Rhodobacter sphaeroides*, the cultured solution was centrifuged to give purple bacteria by decantation. Acetone (200 ml) was added to the purple bacteria (*ca.* 50 g) and the suspension was vigorously stirred under Ar for 10 min. After filtration, the residue was repeatedly extracted with acetone, until the filtrate was colorless (typically, five times). The combined acetone filtrates were concentrated *in vacuo*. To the residue were added aq. 2% HCl (100 ml) and CH_2Cl_2 (100 ml) and the mixture was stirred under Ar for 10 min. The separated CH_2Cl_2 solution was washed with an aq. sat. $NaHCO_3$ solution and H_2O, and dried over Na_2SO_4. After evaporation, the residue was purified by FCC; a dark orange band containing a mixture of several carotenoids was first eluted (CH_2Cl_2) followed by a dark purple band (2–5% Et_2O/CH_2Cl_2). The second fraction was evaporated and recrystallized from MeOH to give bacteriopheophytin-*a* (*ca.* 200 mg); black solids; VIS (CH_2Cl_2) λ_{max}=753 (0.55), 683 (0.11), 530 (0.26), 388 (0.51), 361 (1.00); ^1H NMR ($CDCl_3$) δ=8.96 (1H, s, 5-H), 8.47 (1H, s, 10-H), 8.39 (1H, s, 20-H), 6.08 (1H, s, 13^2-H), 5.17 (1H, t, *J*=7 Hz, phytyl-vinyl-H), 4.50, 4.44 (1H+1H, dd, *J*=7, 12 Hz, $COOCH_2$), 4.27, 4.02 (2H+2H, m, 7-, 8-, 17-, 18-H), 3.84 (3H, s, $COOCH_3$), 3.47 (3H, s, 2-CH_3), 3.43 (3H, s, 12-CH_3), 3.15 (3H, s, $COCH_3$), 2.40–2.55, 2.24–2.36, 2.12–2.21, 2.00–2.09 (2H+2H+1H+1H, m, 8-CH_2, 17-CH_2CH_2), 1.89 (2H, m, phytyl-allyl-H), 1.77, 1.70 (3H+3H, d, *J*=7 Hz, 7-, 18-CH_3), 1.58 (3H, s, phytyl-allyl-CH_3), 1.43–1.53 (3H, m, phytyl-tertiary-H), 1.09 (3H, t, *J*=7 Hz, 8^1-CH_3), 0.93–1.30 (16H, m, phytyl-secondary-H), 0.83, 0.79, 0.78 (6H+3H+3H, d, *J*=7 Hz, phytyl-Me), 0.44, −0.99 (1H+1H, s, NH). MS (FAB) found: *m/z* 888. Calcd for $C_{55}H_{76}N_4O_6$: M^+, 888.

Bacteriopheophytin-*a* (20 mg) in collidine (10 ml) was refluxed under N_2 for 3 h. After evaporation, the residue was purified by FCC (2–3% Et_2O/CH_2Cl_2) and recrystallization from MeOH to give

bacteriopyropheophytin-*a* (18 mg; 96% yield); black solids; VIS (CH_2Cl_2) λ_{max}=754 (0.68), 683 (0.10), 532 (0.27), 386 (0.55), 360 (1.00); ^{1}H NMR ($CDCl_3$) δ=9.00 (1H, s, 5-H), 8.48 (1H, s, 10-H), 8.42 (1H, s, 20-H), 5.25 (1H, t, *J*=7 Hz, phytyl-vinyl-H), 5.11, 4.92 (1H+1H, d, *J*=20 Hz, 13^2-H_2), 4.57, 4.53 (1H+1H, dd, *J*=7, 13 Hz, $COOCH_2$), 4.30 (2H, m, 7-, 18-H), 4.14 (1H, dt, *J*=9, 2 Hz, 17-H), 4.03 (1H, dt, *J*=4, 8 Hz, 8-H), 3.50 (3H, s, 2-CH_3), 3.45 (3H, s, 12-CH_3), 3.17 (3H, s, $COCH_3$), 2.49–2.66, 2.19–2.41, 2.01–2.14 (2H+3H+1H, m, 8-CH_2, 17-CH_2CH_2), 1.94 (2H, br-t, *J*=7 Hz, phytyl-allyl-H), 1.80, 1.73 (3H+3H, d, *J*=7 Hz, 7-, 18-CH_3), 1.64 (3H, s, phytyl-allyl-CH_3), 1.11 (3H, t, *J*=7 Hz, 8^1-CH_3), 0.96–1.54 (19H, m, phytyl-tertiary-H, phytyl-secondary-H), 0.84, 0.792, 0.786 (6H+3H+3H, d, *J*=7 Hz, phytyl-Me), 0.34, −1.05 (1H+1H, s, NH). MS (FAB) found: *m/z* 830. Calcd for $C_{53}H_{74}N_4O_4$: M^+, 830.

To a CH_2Cl_2 (10 ml) solution of bacteriopyropheophytin-*a* (20 mg) was added MeOH (10 ml) of conc. H_2SO_4 (2 ml) at 0°C and the mixture was stirred overnight under N_2. The purple solution was poured into H_2O (150 ml), extracted with CH_2Cl_2, washed with aq. 4% $NaHCO_3$ and brine, and dried over Na_2SO_4. After evaporation, the residue was purified by FCC (6–8% Et_2O/CH_2Cl_2) and washed with hexane and MeOH to give **2b** (11.1 mg; 81% yield); black solids; VIS (CH_2Cl_2) λ_{max}=754 (0.60), 681 (0.094), 628 (0.033), 533 (0.24), 503 (0.06), 466 (0.024), 387 (0.50), 361 (1.00); ^{1}H NMR ($CDCl_3$) δ=8.99 (1H, s, 5-H), 8.47 (1H, s, 10-H), 8.42 (1H, s, 20-H), 5.11, 4.93 (1H+1H, d, *J*=20 Hz, 13^2-H_2), 4.302 (1H, q, *J*=7 Hz, 8-H), 4.300 (1H, dq, *J*=4.5, 7 Hz, 7-H), 4.14 (1H, dt, *J*=8, 2.5 Hz, 17-H), 4.03 (1H, dt, *J*=8.5, 4.5 Hz, 8-H), 3.62 (3H, s, $COOCH_3$), 3.50 (3H, s, 2-CH_3), 3.45 (3H, s, 12-CH_3), 3.16 (3H, s, $COCH_3$), 2.47–2.68, 2.16–2.36 (2H+2H, m, 17-CH_2CH_2), 2.35 (1H, ddq, *J*=4.5, 14, 7 Hz, 8-CH), 2.08 (1H, ddq, *J*=8.5, 14, 7 Hz, 8-CH), 1.80, 1.74 (3H+3H, d, *J*=7 Hz, 7-, 18-CH_3), 1.11 (3H, t, *J*=7 Hz, 8^1-CH_3), 0.36, −1.03 (1H+1H, s, NH). MS (FAB) found: *m/z* 566. Calcd for $C_{34}H_{30}N_4O_4$: M^+, 566.

3.12. *Methyl 3-acetyl-3-devinyl-protopyropheophorbide*-a **2c**

17,18-Oxidation of **2a** gave **2c** (45% yield). Alternatively, oxidation of the 3-(1-hydroxyethyl) group of **1c**[6] gave **2c** (80% yield). FCC (6–15% Et_2O/CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane afforded the pure compound; dark purple solids; VIS (CH_2Cl_2) λ_{max}=647 (0.017), 595 (0.054), 572 (0.073), 527 (0.029), 433 (0.48, sh), 420 (1.00), 393 (21, sh); ^{1}H NMR ($CDCl_3$) δ=10.60 (1H, s, 5-H), 9.89 (1H, s, 10-H), 9.74 (1H, s, 20-H), 5.54 (2H, s, 13^2-H_2), 4.07 (2H, q, *J*=8 Hz, 8-CH_2), 4.02 (2H, t, *J*=8 Hz, 17-CH_2), 3.82 (3H, s, 2-CH_3), 3.80 (3H, s, 12-CH_3), 3.73 (3H, s, $COOCH_3$), 3.64 (3H, s, 18-CH_3), 3.49 (3H, s, 7-CH_3), 3.31 (3H, s, $COCH_3$), 2.94 (2H, t, *J*=8 Hz, 17^1-CH_2), 1.85 (3H, t, *J*=7.5 Hz, 8^1-CH_3), −3.23, −3.83 (1H+1H, s, NH). MS (FAB) found: *m/z* 562. Calcd for $C_{34}H_{34}N_4O_4$: M^+, 562.

3.13. *Methyl 3^1-acetoxyl-mesopyropheophorbide*-a **4**

Acetylation of **1a** by Ac_2O in pyridine gave **4** (45% yield); black solids; VIS (CH_2Cl_2) λ_{max}=647 (0.54), 604 (0.09), 536 (0.11), 505 (0.11), 412 (1.00); ^{1}H NMR ($CDCl_3$) δ=9.65 (1H, s, 5-H), 9.48 (1H, s, 10-H), 8.58 (1H, s, 20-H), 7.2–7.4 (1H, m, 3^1-H), 5.28, 5.12 (1H+1H, d, *J*=18 Hz, 13^2-H_2), 4.50 (1H, m, 18-H), 4.30 (1H, m, 17-H), 3.71 (2H, q, *J*=7 Hz, 8-CH_2), 3.68 (3H, s, $COOCH_3$), 3.65 (3H, s, 12-CH_3), 3.47 (3H, s, 2-CH_3), 3.31 (3H, s, 7-CH_3), 2.50–2.7, 2.30 (3H, s, 3^1-$OCOCH_3$), 2.50–2.7, 2.3–2.36 (2H+2H, m, 17-CH_2CH_2), 2.23 (3H, d, *J*=6 Hz, 18-CH_3), 1.82 (3H, d, *J*=8 Hz, 3^1-CH_3), 1.71 (3H, t, *J*=7 Hz, 8^1-CH_3), −0.68 (1H, br, NH), −1.83 (1H, s, NH). MS (FAB) found: *m/z* 608. Calcd for $C_{36}H_{40}N_4O_5$: M^+, 608.

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