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Transformation of Natural Chlorophyll-*a* into Chlorophyll-*c* Analogs Possessing the 17-Acrylate Residue

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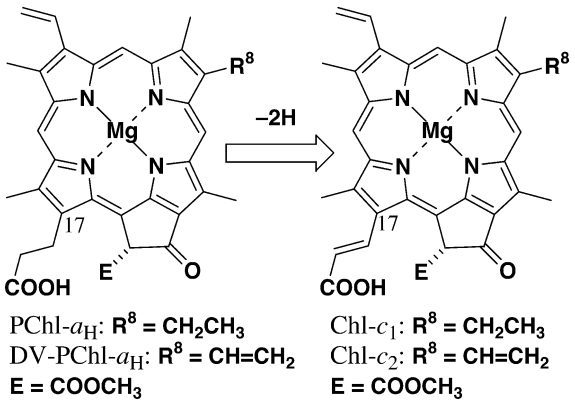
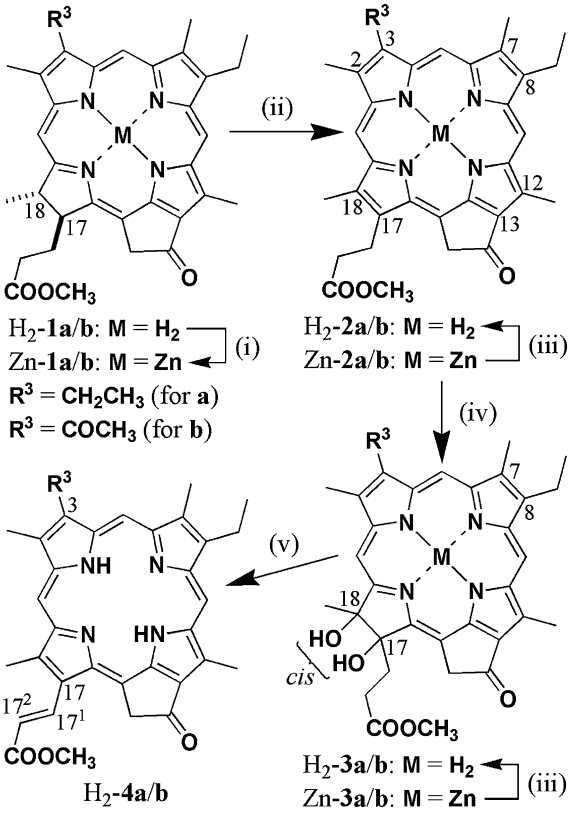
Chlorophyll(Chl)-*a* derivatives possessing C18H–C17H–CH_2–CH_2 were transformed into Chl-*c* analogs possessing C18=C17–CH=CH through dehydrogenation to C18=C17, dihydroxylation to C18(OH)–C17(OH), and double dehydration. This is the first report on the synthesis of the latter porphyrin–acrylate conjugates by modifying natural chlorin–propionate, Chl-*a*.

Chlorophyll(Chl)-*c* is one of the light-harvesting pigments in some oxygenic phototrophs, including brown algae and diatoms.[1] Most Chl-*c* molecules have a fully π-conjugated porphyrin moiety and a free (unesterified) acrylate residue at the 17-position, while other Chls possess a partially reduced porphyrin π-skeleton and a 17-propionate residue esterified with a lipophilic hydrocarbon chain.[2] The unique 17-acrylate residue is proposed to be enzymatically prepared by dehydrogenation of the propionate residue in (divinyl-)protochlorophyllide-*a* [= (DV-)PChl-a_H] as a key intermediate well known for the biosynthesis of other Chl molecules (Scheme 1),[3] but the in vivo transformation pathway has not yet been identified. Here, we first report the synthesis of Chl-*c* analogs consisting of a 17-acrylate-functionalized porphyrin π-conjugate by modifying Chl-*a*. It is noted that Chl-*c* is divided into some molecular species characterized by peripheral substituents: typically, Chl-c_1 for 7-methyl-8-ethyl form and Chl-c_2 for 7-methyl-8-vinyl form.[4] The present Chl-*c* analogs were methyl ester forms of demetallated Chl-c_1 derivatives possessing the 3-ethyl and acetyl groups instead of the 3-vinyl group and lacking the 13^2-methoxycarbonyl group.

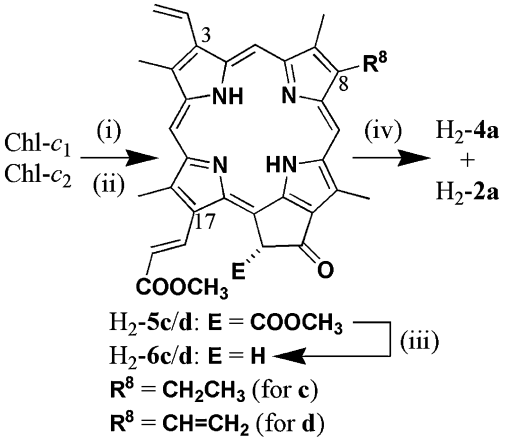
Methyl mesopyropheophorbide-*a* (H_2-**1a**, Scheme 2) was prepared by modifying natural Chl-*a* extracted from a commercially available Spirulina (one of the cyanobacteria) powder according to reported procedures.[5] Oxidation of free-base chlorin H_2-**1a** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) gave a complex mixture of chlorin products, and the corresponding porphyrin H_2-**2a** could not be isolated. After zinc metallation of H_2-**1a** [step (i) in Scheme 2], the resulting Zn-**1a** was readily oxidized by DDQ in acetone [step (ii)][6] to give the desired Zn-**2a**.[7] The facile 17,18-dehydrogenation is ascribable to a decrease in the oxidation potential by the insertion of zinc at the central position. Reaction of Zn-**2a** with osmium tetroxide in the presence of pyridine, followed by treatment with hydrogen sulfide [step (iv)], afforded several products possessing Cβ(OH)–Cβ′(OH).[8] Reverse-phase (RP) HPLC analysis associated with visible absorption and mass spectral data showed nine products, 3 singly dihydroxylated isomers and 6 doubly dihydroxylated isomers: 2,3-, 7,8-, and 17,18-dihydroxychlorins as well as 2,3,7,8- and 2,3,17,18-tetrahydroxyisobacteriochlorins



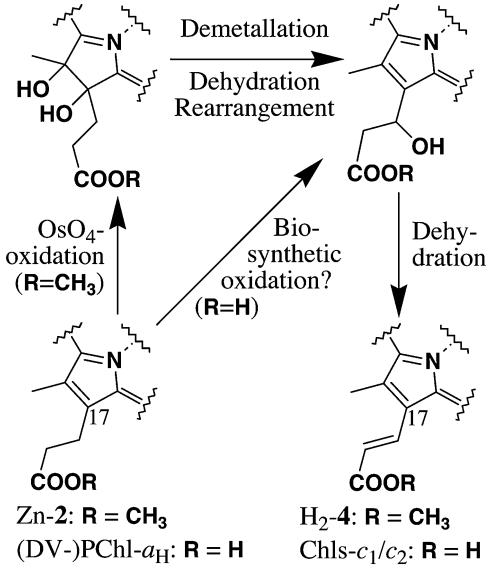
Scheme 1. Proposed biosynthetic route of (divinyl-)protochlorophyllide-*a* [(DV-)PChl-a_H] to chlorophylls-c_1/c_2 (Chls-c_1/c_2).



Scheme 2. Synthesis of chlorophyll-*c* analogs H_2-**4** from chlorophyll-*a* derivatives H_2-**1**: (i) $Zn(OAc)_2 \cdot 2H_2O/CH_3OH$–$CH_2Cl_2$; (ii) DDQ/acetone; (iii) aq. HCl/CH_2Cl_2; (iv) OsO_4, C_5H_5N/CH_2Cl_2 and H_2S; (v) p-$TsOH \cdot H_2O/C_6H_6$, Δ.



Scheme 3. Modification of Chls-c_1/c_2 to H_2-**4a**: (i) aq. HCl/CH_2Cl_2; (ii) CH_2N_2–$(C_2H_5)_2O$/CH_3OH–CH_2Cl_2; (iii) collidine, reflux; (iv) H_2–PtO_2/THF–acetone–C_2H_5OH.



Scheme 4. Probable dehydrogenation pathways of 17-propionate to 17-acrylate residue.

and 7,8,17,18-tetrahydroxybacteriochlorin (two stereoisomers for each tetraol) (Scheme S1 and Figure S1 in ESI). No dihydroxylation occurred at the C12=C13 double bond because of an electron-withdrawing carbonyl group at the 13-position. From the reaction mixture, *cis*-17,18-diol Zn-**3a** was separated by RP-HPLC and treated with an aqueous hydrogen chloride solution [step (iii)] to give H_2-**3a** in 12% isolated yield from Zn-**2a**.[9] The low yield is primarily ascribed to the low regioselectivity in the dihydroxylation to the three Cβ=Cβ′ double bonds of zinc porphyrin Zn-**2a**, C2=C3, C7=C8, and C17=C18. It is noted that free-base form H_2-**2a** prepared by acidic removal of the central zinc of Zn-**2a** was no longer transformed into dihydroxylated (bacterio)chlorin compounds because of its lower oxidizability (vide supra).

Heating a benzene solution of H_2-**3a** and *p*-toluenesulfonic acid (*p*-TsOH) at 50 °C for 6 h [step (v) in Scheme 2][10] consumed all of the starting material and gave a complex mixture of products which lost water, methanol, or water and methanol (Scheme S2 and Figure S2 in ESI). As a hydrophobic fraction with a long retention time, RP-HPLC successfully afforded the doubly dehydrated product H_2-**4a** in 3% isolated yield, which was identified by its ^{1}H NMR, MS, and visible spectra.[11] HPLC analysis showed that no more improvement in the formation of H_2-**4a** could be observed during the reaction. The coupling constant between the C17^1- and C17^2-protons was 16 Hz, indicating the *trans*-form in the 17-acrylate residue. To confirm the molecular structure, H_2-**4a** was alternatively synthesized by modifying natural Chl-*c* extracted from commercially available *Chaetoceros gracilis* cells[4] (Scheme 3). Naturally occurring Chls-c_1/c_2 were transformed into methyl pheophorbides-c_1/c_2 (**5c** and **5d**)[12] by an acid and diazomethane. Pyrolysis of H_2-**5c** and -**5d** in refluxing collidine gave H_2-**6c** and -**6d**, which was hydrogenated on platinum dioxide to afford 3,8-diethyl compound H_2-**4a** after RP-HPLC purification. The product obtained from Chl-*c* was identical to the product from Chl-*a* aforementioned. In the present hydrogenation, H_2-**2a** was obtained as a by-product, so the 3-/3,8-(di)vinyl group(s) of H_2-**6c** and -**6d** was first reduced and the 17-acrylate residue was further reduced to the 17-propionate residue.

Similar to the synthesis of 3-ethylated H_2-**4a**, 3-acetylated H_2-**4b** was produced as follows (Scheme 2). OsO_4 oxidation of Zn-**2b**[13] prepared by DDQ oxidation of Zn-**1b** gave *cis*-17,18-diol Zn-**3b** with 7,8-diol and 7,8,17,18-tetraol. In the dihydroxylation, neither C2=C3 nor C12=C13 reacted because of the 3- and 13-carbonyl groups. A mixture of the two diols separated by flash column chromatography on silica gel was treated with an acid, and RP-HPLC separation afforded H_2-**3b** in 9% isolated yield from Zn-**1b**. Acidic dehydration of H_2-**3b** gave H_2-**4b** (5%) after RP-HPLC separation.[14]

In summary, methyl pyropheophorbide-*a* (R^3 = CH=CH_2 in H_2-**1**),[15] one of the Chl-*a* derivatives possessing the 17-propionate residue on a chlorin π-skeleton, was converted to Chl-*c* analogs H_2-**4a** and -**4b** possessing the 17-acrylate residue on a porphyrin π-skeleton. The proposed dehydrogenation (oxidation and dehydration) mechanism from the 17-CH_2CH_2 moiety to the CH=CH moiety will be useful for elucidating the biosynthetic pathway of (DV-)PChl-a_H to Chls-c_1/c_2 (Scheme 4).

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Supporting Information is available electronically on J-STAGE.

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9 To a solution of Zn-**2a** (17 mg, 28 µmol) in CH_2Cl_2 (10 ml) were added pyridine (400 µl) and OsO_4 (30 mg, 0.12 mmol), and the solution was stirred at room temperature overnight under N_2. MeOH (10 mL) was added and into the solution was bubbled H_2S for 15 min, then the mixture was filtered and purified by FCC (1% MeOH/CH_2Cl_2) and HPLC (Cosmosil 5C_{18}-AR-II 10 $\phi \times 250$ mm, MeOH/H_2O = 87/13, 2.0 mL min^{-1}, retention time; 28 min) to give Zn-**3a**. A CH_2Cl_2 solution (20 mL) of the entire isolated sample of Zn-**3a** was added to the 6% aq. HCl (40 mL) and the mixture was stirred for 5 min, washed with H_2O, sat. aq. $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated. The residue was purified by HPLC (5C_{18}-AR-II 10 $\phi \times 250$ mm, MeOH/H_2O = 95/5, 2.0 mL min^{-1}, retention time: 20 min) to give H_2-**3a** (12%): vis (CH_2Cl_2) λ_{max} = 657 (relative absorbance, 0.26), 599 (0.06), 535 (0.10), 504 (0.10), 407 nm (1.00); ^{1}H NMR ($CDCl_3$): δ 9.46 (1H, s, 5-H), 9.29 (1H, s, 10-H), 8.70 (1H, s, 20-H), 5.43, 5.24 (each 1H, d, J = 20 Hz, 13^1-CH_2), 3.86 (2H, q, J = 8 Hz, 8-CH_2), 3.68 (2H, q, J = 8 Hz, 3-CH_2), 3.56 (3H, s, 7-CH_3), 3.49 (3H, s, 17^2-$COOCH_3$), 3.33 (3H, s, 2-CH_3), 3.27 (3H, m, 12-CH_3), 2.80, 2.02 (each 2H, m, 17-CH_2CH_2), 2.20 (3H, s, 18-CH_3), 1.74 (3H, t, J = 8 Hz, 8^1-CH_3), 1.69 (3H, t, J = 8 Hz, 3^1-CH_3), −1.74 (1H, s, NH) [another NH signal was too broad to be visible.]; MS (LDI) m/z = 583.0 (MH^+).
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11 To a solution of H_2-**3a** (8 mg, 14 µmol) in benzene (15 mL) was added *p*-TsOH·H_2O (4.5 mg, 24 µmol), and the solution was stirred at 50 °C under N_2 for 6 h. After cooling down, the reaction mixture was diluted with CH_2Cl_2 (20 mL), then washed with H_2O, 4% aq. $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated. The residue was purified by HPLC (Cosmosil 5C_{18}-AR-II 10 $\phi \times 250$ mm, MeOH/MeCN = 1/1, 2.5 mL min^{-1}, retention time: 38 min) to give H_2-**4a** (3%): vis (CH_2Cl_2) λ_{max} = 649 (relative absorbance, 0.01), 591 (0.07), 575 (0.07), 529 (0.07), 436 nm (1.00); ^{1}H NMR ($CDCl_3$): δ 10.03 (1H, s, 10-H), 9.88 (1H, s, 5-H), 9.87 (1H, s, 20-H), 9.18 (1H, d, J = 16 Hz, 17-CH), 6.88 (1H, d, J = 16 Hz, 17^1-CH), 5.81 (2H, s, 13^1-CH_2), 4.08 (2H, q, J = 8 Hz, 8-CH_2), 4.07 (3H, s, 12-CH_3), 3.96 (2H, q, J = 8 Hz, 3-CH_2), 3.86 (3H, s, 17^2-$COOCH_3$), 3.72 (3H, s, 7-CH_3), 3.63 (3H, s, 12-CH_3), 3.51 (3H, s, 18-CH_3), 1.88 (3H, t, J = 8 Hz, 8^1-CH_3), 1.84 (3H, t, J = 8 Hz, 3^1-CH_3), −2.24, −3.16 (each 1H, br, NH × 2); HRMS (APCI) found: m/z = 547.2704, calcd for $C_{34}H_{35}N_4O_3$: MH^+, 547.2704.
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14 H_2-**4b**: vis (CH_2Cl_2) λ_{max} = 655 (relative absorbance, 0.01), 597 (0.06), 579 (0.07), 535 (0.05), 439 nm (1.00); ^{1}H NMR ($CDCl_3$): δ 10.53 (1H, s, 5-H), 9.68 (1H, s, 10-H), 9.47 (1H, s, 20-H), 8.54 (1H, d, J = 15 Hz, 17-CH), 6.62 (1H, d, J = 15 Hz, 17^1-CH), 5.10 (2H, s, 13^1-CH_2), 4.12 (3H, s, 2-CH_3), 4.03 (2H, q, J = 8 Hz, 8-CH_2), 3.77 (3H, s, 12-CH_3), 3.70 (3H, s, 17^2-$COOCH_3$), 3.63 (3H, s, 18-CH_3), 3.38 (3H, s, 7-CH_3), 3.33 (3H, s, 3-$COCH_3$), 1.84 (3H, t, J = 8 Hz, 8^1-CH_3) [two NH signals were too broad to be visible.]; HRMS (APCI) found: m/z = 561.2496, calcd for $C_{34}H_{33}N_4O_4$: MH^+, 561.2496.
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