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A mild conversion from 3-vinyl- to 3-formyl-chlorophyll derivatives

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ABSTRACT

The C3-vinyl group of a chlorophyll derivative, methyl pyropheophorbide-*a*, was converted into the formyl group by a novel one-pot reaction with thiophenol at room temperature. The mild reaction can provide insight into development of 'green' catalysts displacing OsO_4 or O_3, and into elucidation of unknown biosynthetic processes of chlorophyll-*d*.

Chlorophyll-*d* (Chl-*d*, **1** see Fig. 1) is a photosynthetic pigment that possesses a formyl group at the C3-position instead of a vinyl group of Chl-*a* (**2**). Chl-*d* absorbs sunlight at a longer wavelength than Chl-*a* does, and functions in the light-harvesting antennas and reaction centers of *Acaryochloris* (*A.*) *marina*, so broad attention has been attracted to its unique structure,[1] the evolution of such photosynthetic systems,[2] and so on. It was reported that *A. marina* had close homologs to Chl-*a* biosynthesis genes,[3] suggesting that Chl-*d* would be synthesized by oxidation of the C3-vinyl of Chl-*a* or chlorophyllide-*a* lacking the phytyl ester (R = H, Fig. 1).

It is also known that Chl derivatives are promising natural photosensitizers applicable to photodynamic therapy[4,5] and dye sensitized solar cells.[6] The C3-substituent of Chls is useful for fine tuning of cell permeability and light absorption. The C3-[1-(1-hexyloxy or 1-octyloxy)ethyl]-derivatives showed relatively better cell permeability than the other compounds.[4] Wittig and Knoevenagel reactions on the C3-formyl group afforded a variety of the C3-ethenyl derivatives with different absorption bands.[7] These compounds required multi-step synthesis from methyl pyropheophorbide-*a* (**3**, see Scheme 1) through C3-(1-bromoethyl)- and formyl-derivatives. The latter oxidation of the C3-vinyl of **3** to formyl group of methyl pyropheophorbide-*d* (**4**) requires hazardous oxidizing reagents such as $KMnO_4$, OsO_4 and O_3.[8–10]

Direct but mild introduction of desired substituents at the C3-moiety is important for Chl chemistry. Among these, a reaction with thiols, good nucleophiles, seems promising. There have been a few reports on the introduction of thio-substituents to the

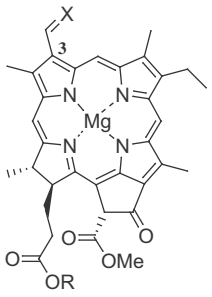
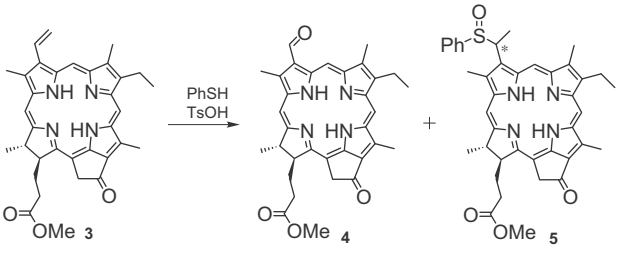


Figure 1. Molecular structures of naturally occurring chlorophyll(Chl)s (R = phytyl). Chl-*d* (**1**): X = O; Chl-*a* (**2**): X = CH_2.



Scheme 1. Conversion of C3-vinyl group of **3** into C3-formyl group of **4**.

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Table 1
Conversion of **3** to **4** and **5**[a] determined by ^{1}H NMR

Thiol	Solvent	**3** (%)	**4** (%)	**5** (%)
PhSH	$CHCl_3$	0	57	15
PhSH	THF	0	51	10
PhSH	MeOH	0	68	25
PhSH	DMSO-d_6	0	47	31
4-MeOPhSH	DMSO-d_6	0	52	36
4-NO_2PhSH	DMSO-d_6	97	3	0
$PhCH_2SH$	DMSO-d_6	90	6	0
PhSH	$CDCl_3$	0	39	14
PhSMe	$CDCl_3$	100	0	0

[a] Reaction of **3** (1 equiv) with thiol (5 equiv) in the presence of $TsOH{\cdot}H_2O$ (4 equiv) at room temperature under N_2 for 18–24 h.

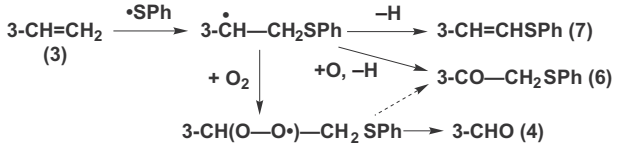
C3^1-position of Chl derivatives.[11,12] We examined one-pot addition of thiols to the C3-vinyl of **3**, and unexpectedly found that the vinyl group was converted into a formyl group to afford **4** (see Scheme 1). Here, we report on the mild conversion method of the vinyl to formyl group at the chlorophyll peripheral position and also the possible route of Chl-*d* biosynthesis.[13]

Typically, compound **3** (10 μmol), prepared from Chl-*a* as previously described,[9] was dissolved in $CHCl_3$. To this solution was added thiophenol (PhSH, 5 equiv) and hydrated *p*-toluenesulfonic acid ($TsOH{\cdot}H_2O$, 4 equiv). The reaction mixture was stirred overnight (18–24 h) in the dark at room temperature under N_2 atmosphere.[14] After work-up, the products were isolated by silica gel flash column chromatography, and analyzed by NMR, MS, and VIS spectroscopies. An initially desired thiol-adduct was obtained in 15% yield as the form of the C3^1-sulfoxide derivative **5**,[15] while the unoxidized Markovnikov adduct of thiophenol (the 3^1-SPh derivative) was rarely obtained. Surprisingly, C3-formylated chlorin **4**[9,10] was obtained through oxidative cleavage of the C3-vinyl group as the main product in 57% yield.[16]

It is noted that alcohol as the solvent improved the yield of compound **4** greatly (nearly 70% in methanol), compared with those in $CHCl_3$, THF and DMSO (ca. 50%) shown in Table 1.[17] In alcohols, the corresponding acetals were partially produced and acidic treatment was necessary for isolation of **4**, where no transesterification at the propionate residue occurred. Conversion of the C3-vinyl group of **3** to the C3-formyl group of **4** was achieved in one-pot reaction without hazardous oxidizing reagents (vide supra); to our knowledge, this is the first report on such an oxidative cleavage of chlorophyll peripheral substituents by a thiol.[18]

The unique oxidation mechanism has not yet been determined, but the radical route was proposed by the following experimental results. The adduct **5** was not a precursor of **4**, because isolated **5** remained unchanged even when stirred with thiophenol and TsOH for 24 h. Compound **5** was also not the oxidation catalyst, because **3** remained unchanged when incubated with **5**. Two by-products were detected from the reaction mixture in $CHCl_3$ and were determined to be C3-$COCH_2$SPh and C3-CH=CHSPh derivatives of **3** (compound **6** and **7**, respectively).[19] The thio-substitution at the C3^2-position indicated that a radical PhS$^{\cdot}$ initially attacked at the C3^2-position of **3** to give 3-CH$^{\cdot}$–CH_2SPh.[20] The resulting C3^1-radical species was oxidatively cleaved to afford **4** and oxidized to produce the above two by-products (Scheme 2). The oxidizing reagent would be oxygen molecules dissolved in solvents for the reaction as shown by time courses of the UV and NMR spectra (data not shown). The above radical mechanism is also supported by the results that the electron-rich thiol (4-MeOPhSH) is more reactive (sensitive to oxidation) than the electron-poor thiol (4-NO_2PhSH) to give **4** smoothly (see Table 1). A similar oxidation reported earlier supports the mechanism too.[21]

The enzyme for oxidation of the C3-vinyl group of Chl-*a* (or chlorophyllide-*a*) to the formyl group has not yet been determined,



Scheme 2. A possible radical mechanism for oxidative cleavage of the C3-vinyl group.

even though the whole genome of *A. marina* producing Chl-*d* has been reported.[3] Kobayashi and his colleagues reported that Chl-*a* was oxidized to give Chl-*d* in less yield by using papain.[22] Recently, Chen and her colleagues suggested that Chl-*a* and O_2 are the biosynthetic precursors of Chl-*d*.[23] Here, we speculate that the C3-vinyl group could be converted to the formyl group by action of thio-functionalized components including a cystein residue in an enzymatic reaction pocket and an oxygen molecule. Oxidoreductase using stable radical species or molecular oxygen, such as cycloxygenase, P450, or peroxidase may also be candidates.

In summary, we have developed a novel, one-pot reaction that can convert the vinyl group of Chl-*a* derivative to a formyl group. Such a mild and efficient conversion from a vinyl group to a formyl group may be included in the yet unclear biosynthesis of Chl-*d*. Our findings can provide insight into elucidation of unknown biosynthetic processes of Chl-*d*, as well as to a novel 'green' catalyst displacing strong oxidizing reagents. Further investigations are now underway.

Acknowledgments

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14. Used nitrogen gas (99.9%) contained O_2 as an impurity, which could influence the reaction.
15. *Methyl 3^1-phenylsulfinyl-mesopyropheophorbide-a* (**5**): A 3^1-epimeric 1:1 mixture; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.55 (1H, s, CH-10), 9.46/9.44 (1H, s, CH-5), 8.54 (1H, s, CH-20), 7.65 (2H, d, J = 8 Hz, *o*-phenyl-H × 2), 7.43 (2H, dt, J = 2.5, 8 Hz, *m*-phenyl-H × 2), 7.36 (1H, dd, J = 2.5, 8 Hz, *p*-phenyl-H), 6.25 (1H, q, J = 8 Hz, CH-3^1), 5.26, 5.12 (2H, 2d, J = 20 Hz, CH_2-13^2), 4.57 (1H, dq, J = 2, 7 Hz, CH-18), 4.30 (1H, dt, J = 7, 2 Hz, CH-17), 3.89/3.88 (3H, d, J = 7 Hz, CH_3-3^2), 3.70 (2H, q, J = 8 Hz, CH_2-8^1), 3.69 (3H, s, CH_3-12^1), 3.61 (3H, s, CH_3-17^5), 3.38/3.37 (3H, s, CH_3-2^1), 3.15/3.14 (1H, s, CH_3-7^1), 2.73–2.52, 2.39–2.17 (4H, m, CH_2-17^1, 17^2), 1.77 (3H, t, J = 7 Hz, CH_3-18^1), 1.69 (3H, t, J = 8 Hz, CH_3-8^2), 0.28, −1.85 (2H, 2*s*, NH × 2); HRMS (FAB, matrix : *m*-nitrobenzyl alcohol) found: m/z 675.3004. Calcd for $C_{40}H_{43}N_4O_4S$: $[M+H]^+$ 675.3005; VIS ($CHCl_3$) λ_{max} 665 nm (relative intensity, 0.53), 412 (1.00); IR (film) ν 1035 cm^{-1} (S═O).
16. *Methyl pyropheophorbide-d* (**4**): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.57 (1H, s, CHO-3), 10.33 (H, s, CH-5), 9.65 (H, s, CH-10), 8.85 (H, s, CH-20), 5.35, 5.20 (2H, 2d, J = 20 Hz, CH_2-13^2), 4.58 (1H, dq, J = 2, 7 Hz, CH-18), 4.38 (1H, dt, J = 7, 2 Hz, CH-17), 3.79 (3H, s, CH_3-2^1), 3.75 (2H, q, J = 8 Hz, CH_2-8^1), 3.74 (3H, s, CH_3-12), 3.62 (3H, s, CH_3-17^5), 3.34 (3H, s, CH_3-7^1), 2.8–2.5, 2.4–2.2 (4H, m, CH_2-17^1, 17^2), 1.85 (3H, d, J = 7 Hz, CH_3-18^1), 1.73 (3H, t, J = 8 Hz, CH_3-8^2), −0.12, −2.03 (2H, 2s, NH × 2); HRMS (FAB, *m*-nitrobenzyl alcohol) found: m/z 551.2656. Calcd for $C_{33}H_{35}N_4O_4$: $[M+H]^+$, 551.2658; VIS ($CHCl_3$) λ_{max} 697 nm (relative intensity, 0.78), 428 (1.00), 384 (0.92). These data were in good agreement with those of **4** previously reported.[9,10]
17. The reaction in MeOH achieved 76% as the best isolated yield.
18. Professor Keely of York Univ. independently found a similar oxidation; Pickering, M. D.; Keely, B. J. Personal communication.
19. **6** (3-$COCH_2$SPh): partial ^{1}H NMR ($CDCl_3$) δ 9.69 (1H, s, CH-5), 9.62 (1H, s, CH-10), 8.75 (1H, s, CH-20), 7.50 (2H, d, J = 7.5 Hz, *o*-phenyl-H × 2), 7.30 (2H, t, J = 7.5 Hz, *m*-phenyl-H × 2), 7.23 (1H, d, J = 7.5 Hz, *p*-phenyl-H), 5.33, 5.19 (2H, 2d, J = 20 Hz, CH_2-13^2), 4.87 (2H, s, CH_2-3^2), −0.11, −2.02 (2H, 2s, NH × 2); MS (MALDI) found: m/z 673.58 ($[M+H]^+$); VIS ($CHCl_3$) λ_{max} = 680 nm. **7** (3-CH═CHSPh): MS (MALDI) found: m/z 656.41 (M^+); VIS ($CHCl_3$) λ_{max} = 672 nm.
20. The reaction with a radical scavenger (BHT) inhibited the production of formylated derivative **4**, which supports the thiyl radical mechanism.
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