Tetrahedron Letters 55 (2014) 725–727

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Radical reaction of chlorophyll derivatives triggered by AIBN

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ARTICLE INFO

Article history:
Received 15 October 2013
Revised 27 November 2013
Accepted 3 December 2013
Available online 8 December 2013

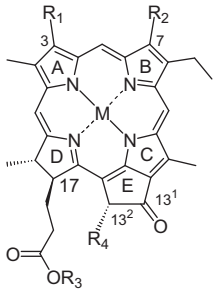
Keywords:
AIBN
Anti-Markovnikov
Chlorophyll
Co-oxidation
Thiol

ABSTRACT

Radical reactions of a C3-vinylated chlorophyll derivative, methyl pyropheophorbide-*a*, which were induced by thiols and the conventional initiator azobisisobutyronitrile (AIBN) were examined in vitro for the first time. Thiyl radicals attacked regioselectively at the sole C3-vinyl group, and the anti-Markovnikov sulfanyl adducts were obtained as major products. The other peripheral substituents, as well as the chlorin macrocycle, remained intact. The AIBN-induced radical reaction competed with co-oxidation that afforded the C3-formyl chlorin. This method can open new routes to derivatization of vinyl chlorins.

Radical reactions are abundant in biochemical processes. Biosynthesis and metabolism of tetrapyrroles also involve radical reactions. Coproporphyrinogen III oxidase generates radical species using *S*-adenosyl-methionine, and catalyzes the oxidative decarboxylation of coproporphyrinogen III to protoporphyrinogen IX.[1] Heme oxygenase utilizes reactive hydroperoxides derived from molecular oxygen to cleave the porphyrin macrocycle at a *meso* position.[2] Chlorophyllide-*a* oxygenase in the synthetic pathway of chlorophyll-*b* (Chl-*b*, Fig. 1) uses tyrosine radical to oxidize the C7-methyl group of chlorophyllide-*a* (Chlide-*a*) or Chl-*a* to a hydroxymethyl group.[3] Molecular oxygen is known to participate in some in vitro degradation reactions of chlorophylls such as allomerization.[4] However, the synthetic potential of radical reactions is far from being fully exploited.

We have found that in the presence of a thiol the C3-vinyl groups of Chl-*a* as well as its derivatives were selectively converted into formyl groups to yield Chl-*d* and its analogues (Scheme 1).[5–7] It is assumed that the formyl chlorins were formed by addition of thiyl radical at the $C3^2$–position of the vinyl double bond, followed by oxidative cleavage of the $C3^1$–$C3^2$ bond. This suggests a new possibility that radical reactions are useful tools for in vitro derivatization of vinyl chlorins, especially at the $C3^2$-position. To date, functionalization at the $C3^2$-position of Chl derivatives has been accomplished by using thallium nitrate,[8] hydroboration,[9] and Corey–Chaykovsky reaction.[10] New synthetic methods and new compounds can offer wider application of chlorophyllous pigments as, for example, photosensitizing drugs for photodynamic therapy. Here we report, for the first time, radical reactions of chlorophyll derivatives with thiols in the presence of a typical initiator, azobisisobutyronitrile (AIBN, Scheme 2).



Substrate	M	R_1	R_2	R_3	R_4
Chl-*a*	Mg	$CH=CH_2$	CH_3	$C_{20}H_{39}$	$COOCH_3$
Chlide-*a*	Mg	$CH=CH_2$	CH_3	H	$COOCH_3$
Chl-*b*	Mg	$CH=CH_2$	CHO	$C_{20}H_{39}$	$COOCH_3$
Chl-*d*	Mg	CHO	CH_3	$C_{20}H_{39}$	$COOCH_3$
1	H_2	$CH=CH_2$	CH_3	CH_3	H

Figure 1. Molecular structures of natural chlorophylls and their derivative **1**.

The vinyl chlorin **1** (112 μmol) was refluxed with 20 equiv of thiophenol (PhSH) and 1 equiv of AIBN in $CHCl_3$. The substrate was completely consumed within 3.5 h and the anti-Markovnikov sulfanyl adduct **2a** ($C3\text{-}CH_2CH_2SPh$) was obtained as the main product in an isolated yield of 38%.[11] The Q_y and the Soret absorption bands of **2a** peaked at 661 and 411 nm in $CHCl_3$, respectively (Fig. 2), in between those of vinyl chlorin **1** (668 and 415 nm) and

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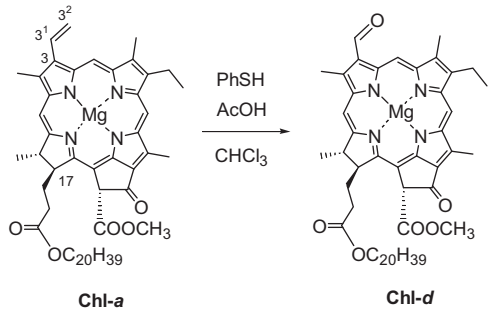
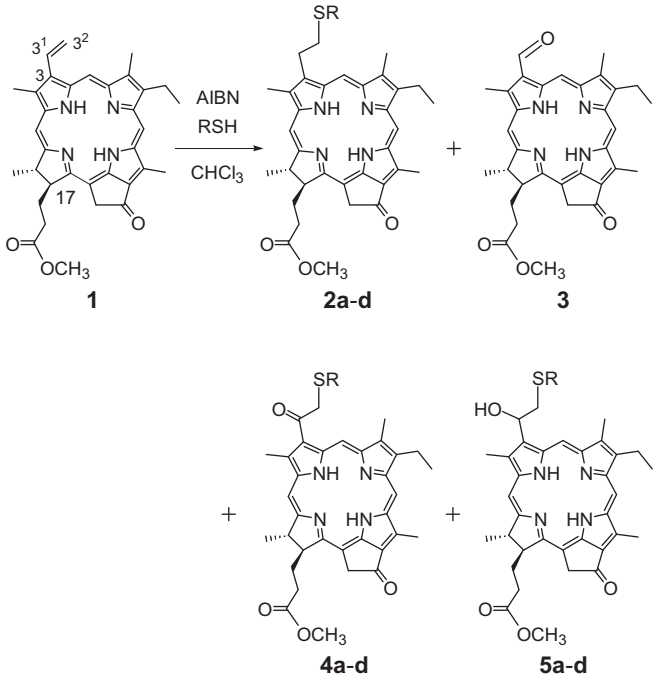
http://dx.doi.org/10.1016/j.tetlet.2013.12.006



Scheme 1. In vitro conversion of Chl-*a* into Chl-*d* using thiophenol and acetic acid.



Scheme 2. Radical reaction of the C3-vinyl double bond of substrate **1** with a thiol and AIBN to give products **2**–**5** (**2a**, **4a**, **5a**: R = PhS–; **2b**, **4b**, **5b**: R = $PhCH_2S$–; **2c**, **4c**, **5c**: R = *p*-$O_2NC_6H_4S$–; **2d**, **4d**, **5d**: R = *p*-$MeOC_6H_4S$–).

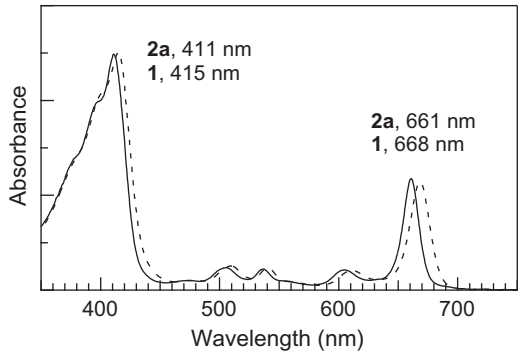


Figure 2. The UV/VIS spectra of the anti-Markovnikov-type sulfanyl adduct **2a** (solid line) and the vinyl chlorin **1** (broken line), recorded in $CHCl_3$ at room temperature. Normalized at the Soret peaks.

methyl mesopyropheophorbide-*a* (C3-CH_2CH_3, 656 and 410 nm in CH_2Cl_2,[12] 656 nm in THF[13]). The characteristic signals (3dd, 6–8 ppm) of the C3-vinyl protons of **1** disappeared, and the phenyl proton signals of **2a** were observed in the range of 7.3–7.7 ppm. We assigned two 2H signals at 4.14 and 3.64 ppm to the $C3^1$- and the $C3^2$-methylene protons, respectively, based on COSY and NOESY measurements and on the data of the C3-CH_2CH_2OH derivative previously reported.[9,10] Compared with **1** and by-products described below, **2a** was much less soluble in MeOH and could be purified by re-precipitation from MeOH.

Note that the major by-product was C3-formyl derivative **3**. To examine the composition of the products, **1** (15 μmol) was reacted as described above and the ^{1}H NMR spectrum of the reaction mixture obtained after workup was analyzed based on the literature data.[5–7] The mixture contained **3** (12%), sulfanyl ketone **4a** (C3-C(=O)CH_2SPh, 5%), and sulfanyl alcohol **5a** (C3-CH(OH)CH_2SPh, 12% as a $C3^1$-racemic mixture), along with the major product **2a** (62%), indicating that co-oxidation occurred simultaneously (Table 1, entry 1). The C3-vinyl group of the substrate was selectively transformed, and during the reaction the products were free from degradations such as hydrogen abstraction at peripheral substituents, ester hydrolysis, and ring opening.

Only a catalytic amount of AIBN (0.05 equiv) was sufficient to add the phenylsulfanyl group to the vinyl double bond of substrate **1** (entry 2). A larger amount of AIBN (1 equiv) did not cause serious degradation. When the reaction was carried out at room temperature, below the 10-h half-life decomposition temperature of AIBN (65 °C), no anti-Markovnikov adduct **2a** was obtained; the oxidized products **3**, **4a**, and **5a** were obtained instead (entry 3), indicating that AIBN was the main initiator triggering formation of anti-Markovnikov adduct **2a**. The system without AIBN also gave co-oxidation products principally (data not shown). The reaction rate depended on the concentrations of both thiol and substrate. The radical reaction of **1** (15 μmol) with 5 equiv of PhSH was very slow, and almost no products were obtained during a 5 h reaction. In contrast, the reaction with 50 equiv of PhSH reached completion within 15 min. Substrate **1** (15 μmol) was completely consumed within 3.5 h when 20 equiv of PhSH was present in the reaction mixture, while the reaction was facilitated in a concentrated solution of **2a** (40 μmol, 2.5 h).

Thioanisole did not give any thiol adduct, indicating that the thiol group is essential for this reaction (data not shown). Benzyl thiol also reacted with **1** to yield the corresponding anti-Markovnikov adduct **2b** in 63–76% (entry 4).[14] A longer time was required for radical addition of this alkyl thiol (9 h) than for that of PhSH (3.5 h), indicating lower reactivity of the alkyl thiol. The reaction of 4-nitrothiophenol was completed within 5 h, and the corresponding $C3^2$-sulfanyl **2c** was obtained in 71–78% yield with little oxidized product (entry 5).[15] It is intriguing that no anti-Markovnikov adduct **2d** was formed by reaction with 4-methoxythiophenol (entry 6). The reaction proceeded rapidly (1 h) and co-oxidation occurred almost exclusively to afford formyl chlorin **3** (48%) and sulfanyl alcohol **5d** (30%) as major products.

We suppose that the simple radical addition competed against the co-oxidation. The co-oxidation preferentially proceeded at relatively low temperature where AIBN cannot trigger radical reactions effectively. Alkyl thiol and 4-nitrothiophenol caused barely any co-oxidation,[5] and the simple radical addition occurred instead. The co-oxidation included anti-Markovnikov addition of thiyl radical to the vinyl double bond in the early stage of the reaction.[5–7] Both 4-nitrothiophenol and 4-methoxythiophenol should attack the $C3^2$-position of the C3-vinyl group of **1**. The difference in reactivity between these thiols might arise from the difference in whether the intermediate radical at the $C3^1$-carbon prefers hydrogen abstraction or addition of molecular oxygen in the subsequent process.

We thus examined AIBN-induced radical reactions of chlorophyll derivative **1** in vitro, for the first time to the best of our knowledge. No serious degradation was induced by thiyl radicals

Table 1
Composition of the reaction products[a]

Entry	R-	AIBN (equiv)	Solv./temp.	Time (h)	 2	 3	 4	 5
1	PhS–	1.0	$CHCl_3$/reflux	3.5	62 (**2a**)	12	5 (**4a**)	12 (**5a**)
2	PhS–	0.05	$CHCl_3$/reflux	5	60 (**2a**)	16	5 (**4a**)	14 (**5a**)
3	PhS–	1.0	$CHCl_3$/rt	5	0 (**2a**)	15	15 (**4a**)	58 (**5a**)
4	$PhCH_2S$–	1.0	$CHCl_3$/reflux	9	63 (**2b**)	0	0 (**4b**)	16 (**5b**)
5	*p*-$O_2NC_6H_4S$–	1.0	$CHCl_3$/reflux	5	71 (**2c**)	2	0 (**4c**)	0 (**5c**)
6	*p*-$MeOC_6H_4S$–	1.0	$CHCl_3$/reflux	1	0 (**2d**)	48	9 (**4d**)	30 (**5d**)

[a] General reaction conditions: 3-vinyl-chlorin **1** (15 μmol), AIBN (1 equiv), and thiol (RSH, 20 equiv) in $CHCl_3$ (10 mL). Yields were determined from the areas of *meso* proton NMR signals specific to respective compounds relative to the total area of the $C13^2$-protons.

and AIBN. The modification occurred at the sole C3-vinyl group, and the rest of the substituent remained unchanged. This simple radical addition competed with co-oxidation. This Letter offers a new, facile method to introduce functional groups at the $C3^2$-position of Chl derivatives. Further study toward the application to photodynamic therapy is now underway.

Acknowledgments

This work was partially supported by a research project of Utsunomiya University Center for Optical Research & Education, and by Grants-in-Aid for Scientific Research (C) (Nos. 21510132 and 25410082) from the Japan Society of the Promotion of Science. We also thank the Collaboration Center for Research and Development of Utsunomiya University, and Professor H. Tamiaki and Dr. T. Mizoguchi (Ritsumeikan University) for their help in analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.12.006.

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11. A typical synthetic method of methyl 3-devinyl-3-[2-(phenylsulfanyl) ethyl]pyropheophorbide-*a* (**2a**) is described below. Methyl pyro pheophorbide-*a* (**1**) was prepared as described in the literature.[16,17] Compound **1** (112.4 μmol, 61.6 mg) was dissolved in dry chloroform ($CHCl_3$, 50 mL). AIBN (1 equiv) and PhSH (20 equiv) were added to the solution, followed by reflux for 5 h. The mixture was washed with water, dried over Na_2SO_4, and evaporated to dryness. Then the residue was redissolved in $C_2H_4Cl_2$. To this solution was added $^tBuNH_2BH_3$ (210 μmol), and the mixture was stirred for 30 min at room temperature under N_2 atmosphere; because polarity of the desired compound **2a** is close to those of the by-products **3** and **4a**, reduction of the $C3^1$=O groups of these by-products facilitated chromatographic separation of **2a**. Aqueous 3% HCl was added to the solution, and the mixture was stirred for 15 min at room temperature, followed by neutralization with aqueous 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by silica gel chromatography (eluent, $C_2H_4Cl_2/Et_2O$ = 100/2), followed by precipitation from MeOH (×3) to afford **2a** as black solid (28.3 mg, 37.8%). The product was analyzed by NMR (Varian VNMR-500), HRMS (Bruker micrOTOF II), and UV/VIS spectroscopy (JASCO V-550). VIS ($CHCl_3$) λ_{max} 661 (ε, 6.3×10^4), 605 (1.1×10^4), 537 (1.2×10^4), 505 (1.2×10^4), 412 nm (1.3×10^5 M^{-1} cm^{-1}); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.51 (1H, s, CH-10), 9.10 (1H, s, CH-5), 8.48 (1H, s, CH-20), 7.64 (2H, d, J = 7 Hz, CH-2′, 6′ of phenyl), 7.42 (2H, t, J = 7 Hz, CH-3′, 5′ of phenyl), 7.36 (1H, t, J = 7 Hz, CH-4′ of phenyl), 5.30, 5.10 (2H, 2d, J = 20 Hz, CH_2-13^2), 4.47 (1H, dq, J = 2, 7 Hz, CH-18), 4.28 (1H, dt, J = 7, 2 Hz, CH-17), 4.14 (2H, m, CH_2-3^1), 3.70 (2H, q, J = 8 Hz, CH_2-8^1), 3.68 (3H, s, CH_3-12^1), 3.64 (2H, m, CH_2-3^2), 3.60 (3H, s, CH_3-17^5), 3.29 (3H, s, CH_3-2^1), 3.19 (3H, s, CH_3-7^1), 2.73–2.51, 2.36–2.22 (4H, 2 m, CH_2-17^1, 17^2), 1.80 (3H, d, J = 7 Hz, CH_3-18^1), 1.70 (3H, t, J = 8 Hz, CH_3-8^2), 0.49, −1.70 (2H, 2s, NH × 2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.2, 173.5, 171.5, 160.0, 155.0, 150.5, 149.0, 145.0, 141.7, 137.6, 137.0, 136.5, 135.9, 135.7, 132.4, 130.3, 129.8, 129.1, 128.1, 126.5, 106.0, 104.1, 95.8, 92.7, 51.7, 51.5, 50.0, 48.0, 35.5, 30.9, 29.8, 26.3, 23.1, 19.4, 17.4, 12.0, 11.21, 11.17. HRMS (APCI) found: m/z 659.3057; calcd for $C_{40}H_{43}N_4O_3S$: MH^+, 659.3056.
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14. Methyl 3-devinyl-3-[2-(benzylsulfanyl)ethyl]pyropheophorbide-*a* (**2b**). VIS ($CHCl_3$) λ_{max} 663 (relative intensity, 0.40), 607 (0.07), 537 (0.08), 507 (0.09), 412 nm (1.00). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.60, 9.26 (2H, 2s, CH-5, 10), 8.57 (1H, s, CH-20), 7.31–7.16 (5H, m, CH of phenyl), 5.28, 5.12 (2H, 2d, J = 20 Hz, CH_2-13^2), 4.50 (1H, dq, J = 2, 7 Hz, CH-18), 4.31 (1H, dt, J = 7, 2 Hz, CH-17), 4.07 (2H, br t, J = 8 Hz, CH_2-3^1), 3.82 (2H, s, CH_2-3^4), 3.73 (2H, q, J = 8 Hz, CH_2-8^1), 3.69, 3.62, 3.26, 3.24 (12H, 4s, CH_3-2^1, 7^1, 12^1, 17^5), 3.19 (2H, br t, J = 8 Hz, CH_2-3^2), 2.78–2.51, 2.37–2.25 (4H, 2 m, CH_2-17^1, 17^2), 1.83 (3H, d, J = 7 Hz, CH_3-18^1), 1.70 (3H, t, J = 8 Hz, CH_3-8^2), −1.73 (1H, 1s, NH) [Another NH was too broad to be observed]. HRMS (APCI) found: m/z 673.3205, calcd for $C_{40}H_{43}N_4O_3S$: MH^+, 673.3212.
15. Methyl 3-devinyl-3-[2-(4-nitrophenylsulfanyl)ethyl]pyropheo-phorbide-*a* (**2c**). VIS ($CHCl_3$) λ_{max} 663 (relative intensity, 0.44), 604 (0.08), 536 (0.08), 505 (0.09), 412 nm (1.00). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.54, 9.15 (2H, 2s, CH-5, 10), 8.50 (1H, s, CH-20), 8.00 (2H, m, CH-3′, 5′ of phenyl), 7.39 (2H, m, CH-2′, 6′ of phenyl), 5.27, 5.12 (2H, 2d, J = 20 Hz, CH_2-13^2), 4.48 (1H, dq, J = 2, 7 Hz, CH-18), 4.30 (1H, dt, J = 8, 2 Hz, CH-17), 4.24 (2H, t, J = 8 Hz, CH_2-3^1), 3.79 (2H, t, J = 8 Hz, CH_2-3^2), 3.71 (2H, q, J = 8 Hz, CH_2-8^1), 3.68, 3.62, 3.32, 3.21 (12H, 4s, CH_3-2^1 7^1, 12^1, 17^5), 2.75–2.52, 2.36–2.26 (4H, 2 m, CH_2-17^1, 17^2), 1.81 (3H, d, J = 7 Hz, CH_3-18^1), 1.71 (3H, t, J = 8 Hz, CH_3-8^2), 0.39, −1.78 (2H, 2s, NH × 2). HRMS (APCI) found: m/z 704.2892, calcd for $C_{40}H_{43}N_4O_5S$: MH^+, 704.2907.
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