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Production of bacteriopurpurin-18 phytyl ester from bacteriopheophytin *a* via allomerization by contact with titanium oxides in the presence of molecular oxygen

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ABSTRACT

Incubation of bacteriopheophytin (BPhe) *a*, which was a demetalated pigment of bacteriochlorophyll *a* in photosynthetic bacteria, in CH_2Cl_2 in the presence of TiO_2 particles with bubbling O_2 in the dark produced a pigment absorbing 814 nm. Detailed characterization of the novel pigment isolated from the CH_2Cl_2 suspension revealed that bacteriopurpurin-18 phytyl ester possessing an anhydride-type six-membered exocyclic E-ring was majorly formed by the treatment with TiO_2 particles under oxygenic conditions. Oxidation of the bacteriochlorin ring in BPhe *a*, namely formations of derivatives of 3-acetyl pheophytin *a* and 3-acetyl protopheophytin *a*, can barely be detected through the conversion processes.

In photosynthesis, cyclic tetrapyrrole pigments such as chlorophylls (Chls) and bacteriochlorophylls (BChls) play essential roles in the processes of light-harvesting and charge separation. Chlorin-type cyclic tetrapyrroles (17,18-dihydroporphyrins) are photofunctional moieties of photosynthetic pigments such as Chls *a* and *b* in oxygenic photosynthetic organisms. In contrast, bacteriochlorin-type pigments (7,8,17,18-tetrahydroporphyrins) function as light-harvesting and charge separation in bacterial photosynthesis.[1,2] Fig. 1A shows the molecular structure of BChl *a*, which is a major pigment in purple photosynthetic bacteria. Owing to reduction of the bonds between the 7- and 8-positions as well as between 17- and 18-positions in the cyclic tetrapyrrole ring, BChl *a* exhibited an intense Qy absorption band in the near-infrared (NIR) region, whose peak position is red-shifted by approximately 100 nm compared with chlorin-type pigments.[1–3] Such unique spectral features of bacteriochlorin-type photosynthetic pigments allow us to utilize them for developments of NIR-responsive pigments, which have attracted considerable attentions in the research area of life sciences and materials chemistry.[4–7] In particular, BChl *a* and its derivatives have been fascinated as photosensitizers in photodynamic therapy (PDT) because of their efficient absorption abilities of NIR light that can penetrate tissues.[5–13] Henderson et al. first attempted to use BChl *a* as PDT sensitizers to tumors.[8] However, BChl *a* was degraded *in vivo* and such degradation affected PDT activities. To improve stabilities and PDT activities of BChl *a* derivatives, substitution of central metals and peripheral groups in the bacteriochlorin macrocycle has been extensively examined.[9–13]

Photosynthetically active chlorophyllous pigments have a five-membered exocyclic E-ring with a 13^2-methoxycarbonyl group. The change of the E-ring to six-membered rings such as anhydride and imide-type exocyclic rings, namely conversions to purpurin- and purpurinimide-type pigments, is one of the promising strategies to regulate optical and physicochemical properties of chlorophyllous pigments.[14–20] Pandey and coworkers have developed various bacteriopurpurin- and

bacteriopurpurinimide-type functional pigments from natural BChl *a* and demonstrated their utilities as stable PDT photosensitizers that can work effectively by excitation of NIR light.[21–27]

Bacteriopurpurin-18 (the molecular structure is shown in Fig. 1B) is known to be a key compound in the synthesis of bacteriopurpurinimides. Bacteriopurpurin-18 is generally synthesized under strong alkaline conditions with O_2 from bacteriopheophytin (BPhe) *a* and its derivatives. In this process, however, hydrolysis of an ester group at the 17-propionate residue of chlorophyllous pigments is inevitable and thus re-esterification is required in many cases. Additionally, undesirable side-reactions sometimes occur in the step of re-esterification in the synthesis of purpurin-type pigments.[28] In such situations, we previously found that pheophorbide *a* methyl ester consisting of the chlorin π-macrocycle was converted to purpurin-18 methyl ester by contact with TiO_2 particles under oxygenic conditions in the dark.[20] This reaction encourages us to attempt one-step conversion from BPhe *a* to bacteriopurpurin-18 phytyl ester under mild conditions. In this study, we examine behaviors of BPhe *a* by contact with TiO_2 particles with O_2-bubbling in the dark and characterize a major product isolated from the reaction mixture.

BPhe *a* was prepared by demetalation of natural BChl *a*, which was extracted from a purple photosynthetic bacterium *Rhodobacter sphaeroides*, under acidic conditions.[29] Anatase-type TiO_2 particles (diameter, 5 μm; purity, 99.9%; Wako Chemical Industries, Ltd.) were added to a dichloromethane solution of BPhe *a*, followed by stirring with bubbling O_2 in the dark at 25 °C. Visible absorption spectra of CH_2Cl_2 solutions after removal of TiO_2 particles by filtration were measured at a regular interval. Spectral measurements were performed within ca. 4 h, since CH_2Cl_2 significantly decreased by O_2-bubbling for a long period.

Fig. 2 compares visible absorption spectra of BPhe *a* incubated for 4 h with TiO_2 particles in CH_2Cl_2 by bubbling O_2 in the dark (thick solid curve) with those in control experiments (thin solid and broken curves) and before incubation (dotted curve). BPhe *a* before incubation exhibited a Qy

absorption band at 753 nm in CH_2Cl_2 (dotted curve). When a CH_2Cl_2 suspension containing BPhe *a* was bubbled by O_2 in the presence of TiO_2 particles, a new Qy absorption band appeared at 814 nm (thick solid curve), indicating that a new pigment absorbing 814-nm NIR light (hereafter denoted **1**) was produced from BPhe *a* under the oxygenic conditions. On the contrary, no absorption band was detected above 800 nm by bubbling N_2 in the presence of TiO_2 particles (thin solid curve) and by bubbling O_2 without TiO_2 particles (broken curve). These indicate that TiO_2 particles were necessary for this conversion under the oxygenic conditions. It is worth noting that absorbance increase between 600 and 700 nm, which is derived from formation of the chlorin and porphyrin π-macrocycles by oxidation of the bacteriochlorin ring, barely increased under the present conditions.

To characterize the product **1** absorbing 814 nm in detail, **1** was purified by preparative HPLC using a reverse-phase column ($5C_{18}$-AR-II, Nacalai Tesque) with methanol/chloroform (8/2) after filtration of the reaction suspension and evaporation under reduced pressure. This pigment had the molecular ion peak at *m*/*z* 860.5429 in the high-resolution mass spectrum, which was identical to the molecular weight of bacteriopurpurin-18 phytyl ester (860.5452). Fig. 3 depicts ^{1}H NMR spectra of **1** and intact BPhe *a*. BPhe *a* showed two singlet peaks at 6.09 and 3.85 ppm, which were ascribed to 13^2-H and 13^4-CH_3, respectively (Fig. 3A). These signals disappeared in the ^{1}H NMR spectrum of **1** (Fig. 3B), indicating the lack of both the proton and the methoxycarbonyl group at the 13^2-position in this compound. The 17-H signal of **1** was shifted to the lower field by approximately 1.1 ppm compared with that of BPhe *a*. Such a large shift is characteristic of (bacterio)purpurin-18 type pigments.[20,26] Given the molecular structure of bacteriopurpurin-18 phytyl ester (Fig. 1B), all the signals in the ^{1}H NMR spectrum of **1** can be assigned as described in Supplementary Data. Fig. 4 shows a FTIR spectrum of the compound **1**. This pigment exhibited a vibrational band at 1720 cm^{-1}, which can be attributed to the anhydride group in the exocyclic E-ring of (bacterio)purpurin-type chlorophyllous pigments, in the lower-wavenumber side of the stretching vibrational band of the

17^2-C=O in the ester moieties at 1746 cm^{-1}. The stretching vibrational band of the 13-keto group in intact BPhe *a* around 1700 cm^{-1} disappeared in the FTIR spectrum of **1**. The bands at 1670-cm^{-1} and around 1600–1620 cm^{-1} were ascribable to the C=O stretching vibrational band in the 3-acetyl group and skeletal vibrational bands of the bacteriochlorin π-macrocycle, respectively. These characterizations proved that the pigment **1**, which was mainly produced by contact with TiO_2 particles in the presence of O_2, was bacteriopurpurin-18 phytyl ester. The yield of **1** was estimated to be 7% in the present reaction, which was lower than the conversion efficiency from pheophorbide *a* methyl ester to purpurin-18 methyl ester (32%).[20] One possible reason for the difference in the conversion efficiency might be adsorption behaviors on TiO_2 particles; more hydrophobic bacteriopheophytin *a* phytyl ester tended to be less adsorbed on TiO_2 surfaces. The adsorption abilities of chlorophyllous pigments are responsible for the yields in this type of reaction, since the contact of substrates on TiO_2 surfaces is necessary for the conversion into (bacterio)purpurin-type pigments.

In the conventional synthesis of purpurin-18 and bacteriopurpurin-18, the enolate anions were generated from chlorophyllous pigments by subtraction of the 13^2-proton in the E-ring in the action of hydroxide ions, followed by oxidation with O_2 to form the six-membered anhydride ring.[30–32] The terminal and/or bridging hydroxides[33–35] would be responsible for the formation of the enolate anions in the early stage of the present conversion, followed by oxidation in the same manner as the conventional conversions. Interactions of the 13-keto group in the E-ring of chlorophyllous pigments with TiO_2 surfaces[36] might contribute to smooth formation and/or stabilization of the enolate anions.

The present conversion from BPhe *a* to bacteriopurpurin-18 phytyl ester proceeded under mild conditions without cleavage of the phytyl ester at the 17-propionate residue. This is in sharp contrast to the conventional synthesis of bacteriopurpurin-18 derivatives under strong alkaline

conditions, accompanying inevitable hydrolysis of ester moieties. In addition, a work-up procedure in this conversion, namely filtration and evaporation after the reactions, is significantly facile compared with that in the conventional synthesis. This reaction, therefore, would be advantageous from the viewpoints of green chemistry and will be useful for developments of NIR-light responsive photofunctional pigments from BChl *a* in photosynthetic bacteria.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at.

REFERENCES

1. Scheer, H. In *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and*

Applications; Grimm, B., Porra, R. J., Rüdiger, W., Scheer, H., Eds.; Springer: Dordrecht, 2006; pp 1–26.

2. Tamiaki, H.; Kunieda, M. In *Handbook of Porphyrin Science*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; World Scientific: Singapore, 2011; Vol.11, pp 223–290.
3. Linnanto, J.; Korppi-Tommola, J. *Phys. Chem. Chem. Phys.* **2006**, *8*, 663.
4. Wang, X.-F; Tamiaki, H. *Energy Environ. Sci.* **2010**, *3*, 94.
5. Sternberg, E. D.; Dolphin, D. *Tetrahedron* **1998**, *54*, 4151.
6. Nyman, E. S.; Hynninen, P. H. *J. Photochem. Photobiol. B: Biol.* **2004**, *73*, 1.
7. Chen, Y.; Li, G.; Pandey, R. K. *Curr. Org. Chem.* **2004**, 8, 1105.
8. Henderson, B. W.; Sumlin, A. B.; Owczarczak, B. L.; Dougherty, T. J. *J. Photochem. Photobiol. B: Biol.* **1991**, *10*, 303.
9. Kessek, D.; Smith, K. M.; Pandey, R. K.; Shiau, F.-Y.; Henderson, B. *Photochem. Photobiol.* **1993**, *58*, 200.
10. Fiedor, J.; Fiedor, L.; Kammhuber, N.; Scherz, A.; Scheer, H. *Photochem. Photobiol.* **2002**, *76*, 145.
11. Vakrat-Haglili, Y.; Weiner, L.; Brumfeld, V.; Brandis, A.; Salomon, Y.; Mcllroy, B.; Wilson, B. C.; Pawlak, A.; Rozanowska, M.; Tadeusz, S.; Scherz, A. *J. Am. Chem. Soc.* **2005**, *127*, 6487.
12. Limantara, L.; Koehler, P.; Wilhelm, B.; Porra, R. J.; Scheer, H. *Photochem. Photobiol.* **2006**, *82*, 770.
13. Dandler, J.; Wilhelm, B.; Scheer, H. *Photochem. Photobiol.* **2010**, *86*, 182.
14. Hoober, J. K.; Sery, T. W.; Yamamoto, N. *Photochem. Photobiol.* **1998**, *48*, 579.
15. Lee, S. J. H.; Jagerovic, N.; Smith, K. M. *J. Chem. Soc. Perkin Trans. 1* **1993**, 2369.
16. Kozyrev, A. N.; Zheng, G.; Lazarou, E.; Dougherty, T. J.; Smith, K. M.; Pandey, R. K. *Tetrahedron Lett.* **1997**, *38*, 3335.

17. Zheng, G.; Potter, W. R.; Sumlin, A.; Dougherty, T. J.; Pandey, R. K. *Bioorg. Med. Chem.* **2000**, *10*, 123.

18. Pandey, S. K.; Sajjad, M.; Chen, Y.; Pandey, A.; Missrt, J. P.; Batt, C.; Yao, R.; Nabi, H. A.; Oseroff, A. R.; Pandey, R. K. *Bioconjugate Chem.* **2009**, *20*, 274.

19. Wang, X.-F.; Wang, L.; Tamai, N.; Kitao, O.; Tamiaki, H.; Sasaki S. *J. Phys. Chem. C* **2011**, *115*, 24394.

20. Saga, Y.; Otono, T. *Chem. Lett.* **2012**, *41*, 360.

21. Kozyrev, A. N.; Zheng, G.; Zhu, C.; Dougherty, T. J.; Smith, K. M.; Pandey, R. K. *Tetrahedron Lett.* **1996**, *37*, 6431.

22. Chen, Y.; Graham, A.; Potter, W.; Morgan, J.; Vaughan, L.; Bellnier, D. A.; Henderson, B. W.; Oseroff, A.; Dougherty, T. J.; Pandey, R. K. *J. Med. Chem.* **2002**, *45*, 255.

23. Kozyrev, A. N.; Chen, Y.; Goswani, L. N.; Tabaczynski, W. A.; Pandey. R. K. *J. Org. Chem.* **2006**, *71*, 1949.

24. Gryshuk, A. L.; Chen, Y.; Potter, W.; Ohulchansky, T.; Oseroff, A.; Pandey, R. K. *J. Med. Chem.* **2006**, *49*, 1874.

25. Gryshuk, A. L.; Chen, Y.; Goswami, L. N.; Pandey, S.; Missert, J. R.; Ohulchanskyy, T.; Potter, W.; Prasad, P. N.; Oseroff, A.; Pandey, R. K. *J. Med. Chem.* **2007**, *50*, 1754.

26. Goswani, L. N.; Chen, Y.; Missert, J.; Li, G.; Pallenberg, A.; Pandey, R. K. *Heterocycles* **2007**, *71*, 1929.

27. Chen, Y.; Potter, W. R.; Missert, J. R.; Morgan, J; Pandey, R. K. *Bioconjugate Chem.* **2007**, *18*, 1460.

28. Kozyrev, A. N.; Alderfer, J. L.; Dougherty, T. J.; Pandey, R. K. *Chem. Commun.* **1998**, 1083.

29. Saga, Y.; Miura, R.; Sadaoka, K.; Hirai, Y. *J. Phys. Chem. B* **2011**, *115*, 11757.

30. Hynninen, P. H.; Leppäkases, T. S.; Mesilaakso, M. *Tetrahedron Lett.* **2006**, *47*. 1663.

31. Hynninen, P. H. In *Chlorophylls*; Scheer, H., Ed.; CRC Press: Boca Raton, 1991; pp 145–209.

32. Hynninen, P. H.; Hyvärinen, K. *J. Org. Chem.* **2002**, *67*, 4055.

33. Hadjiivanov, K. I.; Klissurski, D. G. *Chem. Soc. Rev.* **1996**, *25*, 61.

34. Connor, P. A.; Dobson, K. D.; McQuillan, A. J. *Langmuir* **1999**, *15*, 2402.

35. Sano, K.; Shiba, K. *J. Am. Chem. Soc.* **2003**, *125*, 14234.

36. Nakajima, K.; Noma, R.; Kitano, M.; Hara, M. *J. Phys. Chem. C* **2013**, *117*, 16028.

FIGURE CAPTION

Fig. 1 (A) Molecular structures of BChl *a* (M = Mg) and BPhe *a* (M=2H). (B) Molecular structures of bacteriopurpurin-18 (R = H) and bacteriopurpurin-18 phytyl ester (R = phytyl).

Fig. 2. Visible absorption spectra of CH_2Cl_2 solutions containing BPhe *a* after incubation for 4 h by bubbling O_2 with TiO_2 particles (thick solid curve), by bubbling N_2 with TiO_2 particles (thin solid curve), by bubbling O_2 without TiO_2 particles (broken curve), and before incubation (dotted curve). Spectra were normalized at the Soret peaks.

Fig. 3. ^1H NMR spectra of BPhe *a* (A) and the product **1** isolated from a CH_2Cl_2 solution after incubation with TiO_2 particles and O_2 (B) in $CDCl_3$ between 3 and 7 ppm.

Fig. 4. FTIR spectrum of the product **1** isolated from a CH_2Cl_2 solution after incubation with TiO_2 particles and O_2 in CH_2Cl_2 between 1570 and 1850 cm^{-1}.

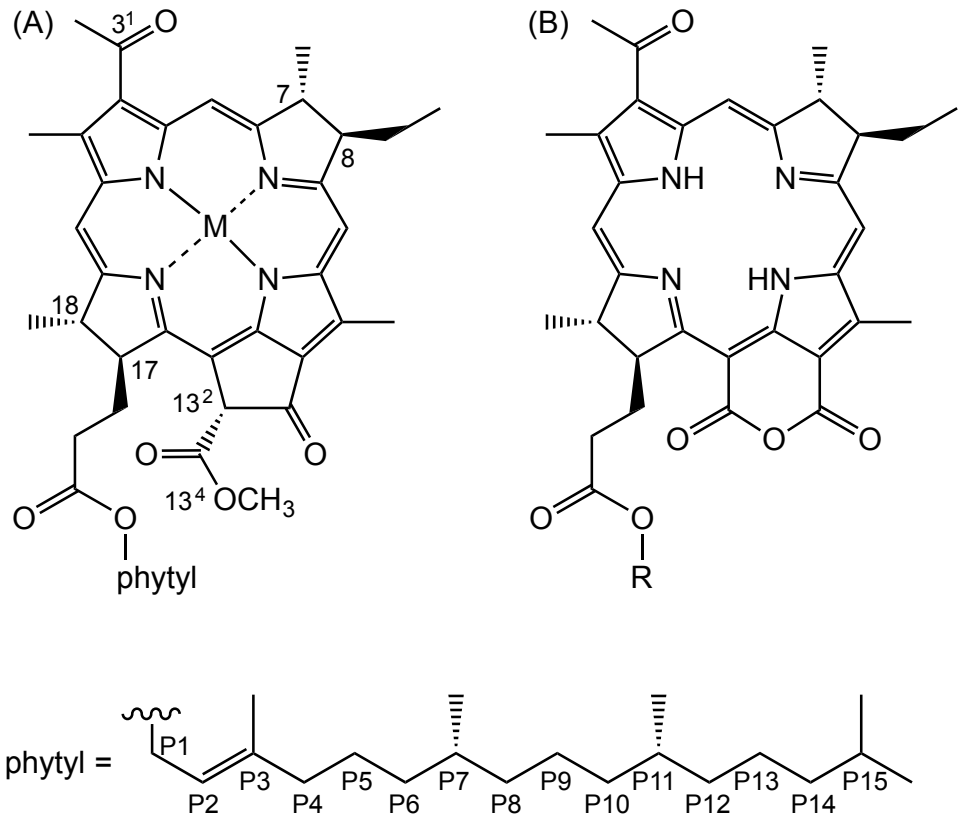

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Fig. 1

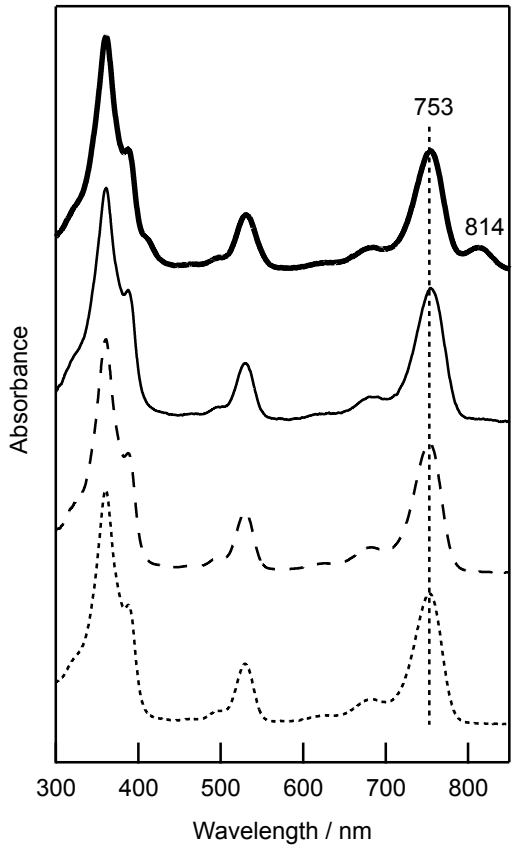

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Fig. 2

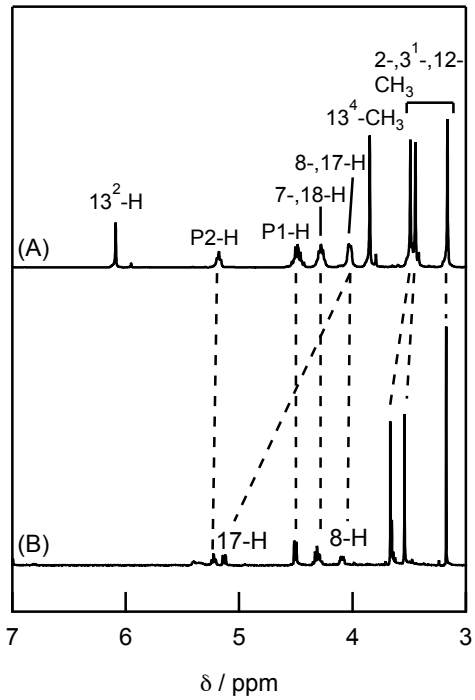

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Fig. 3

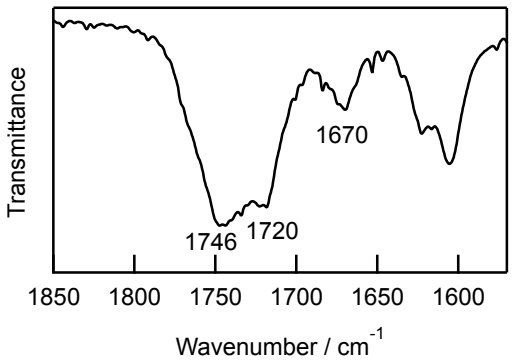

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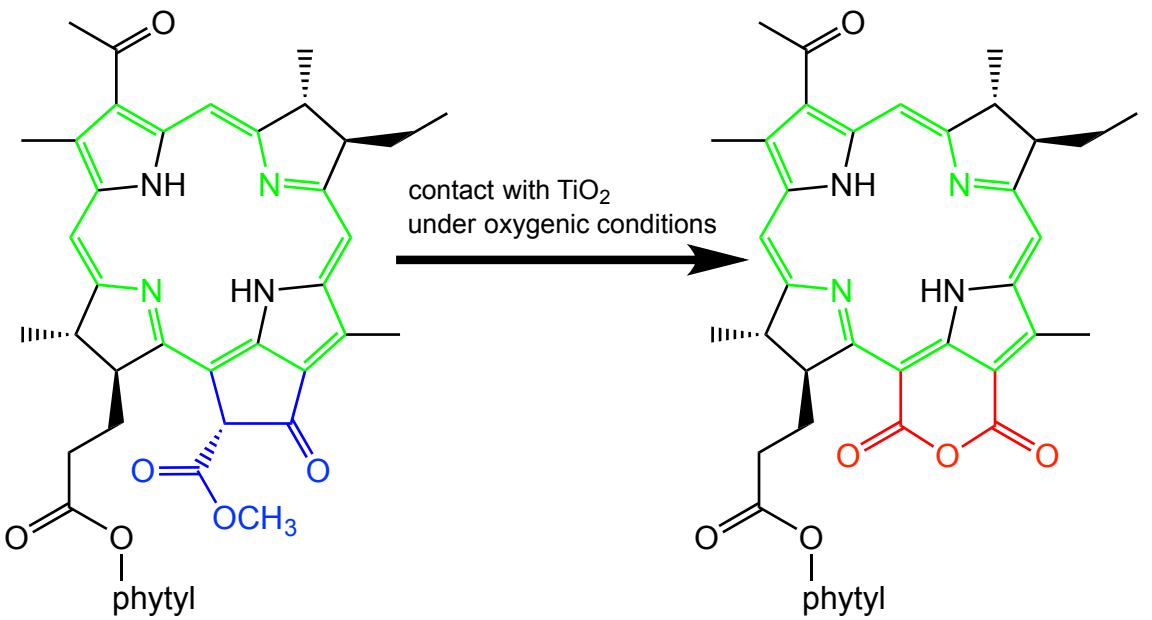
Fig. 4



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Graphical abstract