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Gallium(III) complexes of methyl pyropheophorbide-*a* as synthetic models for investigation of diastereomerically controlled axial ligation towards chlorophylls

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Abstract—Gallium(III) chlorins possessing a series of axial ligands were synthesized as model compounds of natural chlorophylls. A pair of diastereomers arising from the fifth axial coordination onto the asymmetric chlorin π-macrocycle could be discriminated in a solution by both ^{1}H- and ^{13}C NMR spectroscopies.

Chlorophylls (Chls) are magnesium complexes of asymmetric cyclic tetrapyrroles and main dye molecules of natural photosynthetic systems.[1] Several X-ray crystal structure analyses of protein–Chl complexes have shown that all Chls are fixed in proper locations by the fifth coordination to their central magnesium,[2] which enables the efficient and rapid light-harvesting, energy transfer and charge transfer processes. Recently, we and Balaban et al. reported that one of the two sides in Chls, the opposite direction of the 17-propionate, is a favourable asymmetric site for the fifth ligation (α-ligated complex, Fig. 1).[2–4] To investigate the coordination chemistry of Chls in solution using the ^{1}H NMR technique, Mg(II) and Zn(II) chlorins have already been studied.[5,6] In these cases, two possible stereoisomers arising from a fifth ligation have never been distinguished due to the rapid equilibrium between coordination and dissociation of the ligand on a NMR time-scale. Thus, studies concerning such diastereomerically selective coordination of Chls were limited to their crystallographic analyses in solid states as well as their theoretical modelling in silicon.

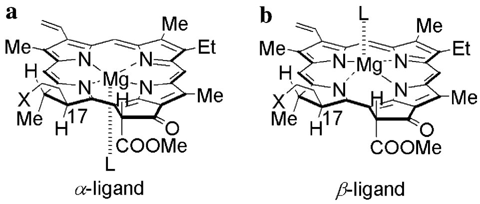

Figure 1. Molecular structures of fifth-coordinated chlorophyll-*a*. (a) α- and (b) β-ligated complexes. X = COO-phytyl, L = axial ligand.

During our synthetic study on metallochlorins to mimic self-aggregates in main light-harvesting antennas of green photosynthetic bacteria,[7] we found that fifth-coordinated Ga(III) chlorin is a good model to investigate the axial ligation towards its asymmetric π-macrocycle plane. Preparation of Ga(III) porphyrin derivatives and their physical properties have already been reported,[8] and recent examples include not only some basic study[9–11] but also analytical and medicinal applications.[12,13] However, to our best knowledge, only one Ga(III) chlorin having an asymmetric plane is available in the literature,[14] where no coordination event has been described. Here, we report for the first time discrimination of two diastereomers of Chl derivatives having an axial fifth ligand in solution by means of the NMR technique. Ga(III) chlorins possessing a series of axial ligands are shown to be new useful models for studying the coordination chemistry of Chls.

Ga(III) chlorin **1** was prepared from methyl pyropheophorbide-*a*[15] as shown in Scheme 1. Mild reaction

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Keywords: Chlorin; Coordination; Gallium; Photosynthesis; Porphyrin.

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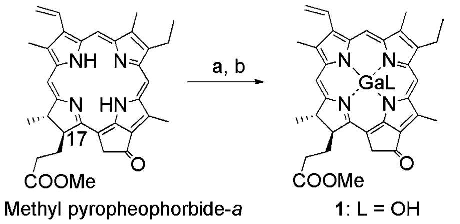


Scheme 1. Reagents and condition: (a) $GaCl_3$, $CHCl_3$–MeOH, reflux; (b) 6% H_2O–Al_2O_3, 5% MeOH–CH_2Cl_2.

conditions for the gallium insertion to the above metal-free chlorin (step (a) in Scheme 1) were selected to yield the corresponding Ga(III)Cl complex. Subsequent anion-exchange reaction was achieved by passing through a basic alumina column deactivated by 6% (w/w) water[16] to obtain Ga(III)(OH) complex **1**.[17] ^1H NMR spectrum of the product in $CDCl_3$ showed a pair of signals for both chlorin moiety and axial OH ligand (−5.11 and −5.29 ppm at 20 °C). Large upfield shifts of the hydroxyl hydrogen are ascribable to the shielding effect of chlorin π-macrocycle. When measured at a lower temperature (−50 °C), each signal became sharper as can be seen in Figure 2, while no coalescence was observed at a higher temperature examined up to 50 °C. This observation indicates that the two species are relatively stable in this solution state. A set of OH signals showed 1 equiv of integration area compared to the chlorin signals. It is noteworthy that the ratio of two species is inequivalent, ca. 1:1.3. Such an inequivalent set of signals was also observed in the ^{13}C NMR spectrum measured in $CDCl_3$.

On the other hand, FAB-MS spectrum of Ga(III)(OH) chlorin **1** using *m*-nitrobenzyl alcohol (NBA) as a matrix gave signals corresponding to $[M-OH]^+$, $[M]^+$ and $[M-OH+NBA]^+$ at 615 (relative intensity, 100), 632 (18) and 767 (53), respectively. This result shows that one hydroxy group was bonded to Ga(III) chlorin in the product and partially replaced by NBA. The ligand exchange by another alcohol was also confirmed by ^1H NMR spectroscopy. When methanol was added to **1** in $CDCl_3$, new peaks appeared at −0.39 and −0.45 ppm (Ga–OMe) together with the corresponding chlorin signals, while the unreacted peaks of **1** were retained (see Supplementary data). To further investigate the ligand exchange, a $CDCl_3$ solution of **1** was treated with *p*-bromophenol. It was found that the hydroxy group of **1** was almost quantitatively replaced by the *p*-bromophenoxide group until 1 equiv of *p*-bromophenol was added. Even in the presence of excess *p*-bromophenol (10 equiv), the integration area of a pair of coordinated ligands remained just 1 equiv to those of the chlorin peaks. Since all three kinds of ligands (HO^-, CH_3O^- and p-$BrC_6H_4O^-$) brought similar ^1H NMR spectra showing a pair of signals in $CDCl_3$, it seems reasonable to think that the relatively stable fifth ligation towards the asymmetrical faces caused distinguishable stereoisomers.

In order to unambiguously prove the assumption that the two stereoisomers of the fifth-ligated complexes of Ga(III) chlorins **1**–**3** (Fig. 3) were discriminated in their proton signals, the corresponding Ga(III) porphyrins were prepared and expected to form a pair of enantiomers in their fifth-coordinated state as illustrated in Figure 4, which were indistinguishable by the standard ^1H NMR technique. After gallium insertion of methyl protopyropheophorbide-*a*[18] (methyl 17,18-dehydropyropheophorbide-*a*) and ligand exchange through an alumina column using methanolic solvent, Ga(III) porphyrin complex was obtained as a mixture of **5** and **6** having OH and OMe group as an axial ligand, respectively; δ (OH) = −7.72 and δ (OMe) = −2.29 ppm. Further ligand exchange of the mixture was performed to simplify the spectrum for direct comparison between Ga(III) chlorin and porphyrin. Benzyl thiolate was selected as a stronger coordinate as reported earlier.[19,20] Thus, Ga(III) chlorin **4** and Ga(III) porphyrin **7** were prepared by treatment of chlorin **1** or a mixture of porphyrins **5**/**6** with phenylmethane thiol (benzyl thiol) in CH_2Cl_2.[21]

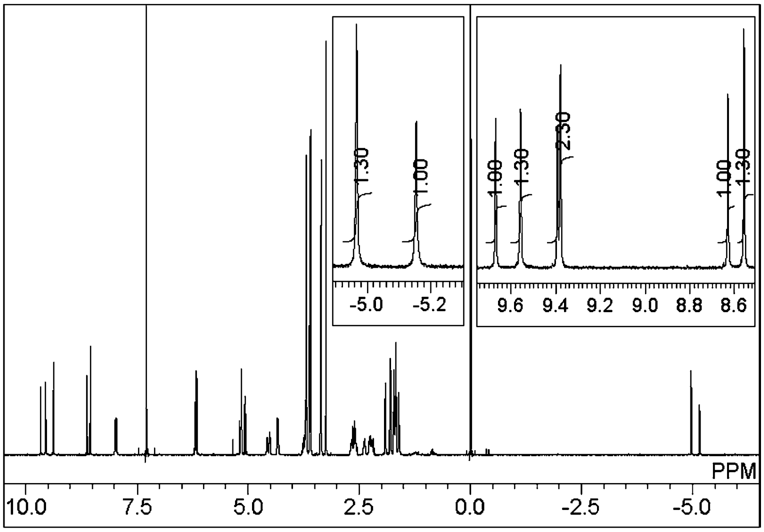
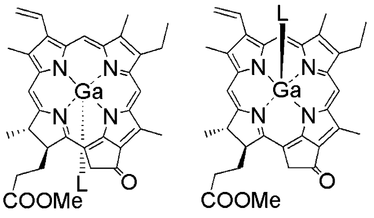
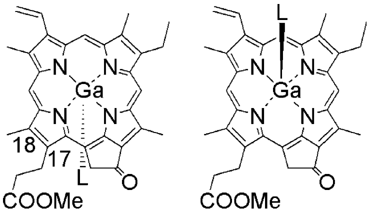


Figure 2. ^1H NMR spectrum of Ga(III)(OH) chlorin **1** in $CDCl_3$ at −50 °C.



1: L = OH
2: L = OMe
3: L = $OC_6H_4Br(p\text{-})$
4: L = SCH_2Ph

Figure 3. Molecular structures of fifth-coordinated Ga(III) chlorins **1**–**4** represented as a pair of diastereoisomers.



5: L = OH
6: L = OMe
7: L = SCH_2Ph

Figure 4. Molecular structures of Ga(III) porphyrins **5**–**7** represented as a pair of enantiomers.

Figure 5 compares the ^{1}H NMR spectra of **4** and **7** in $CDCl_3$. Each proton of **4** shows a pair of signals with a ratio of 1:1.5, and the fifth ligand shows the characteristic upfield shift with 1 equiv of integration area as represented by marks (•) (see Fig. 5a). Similar pairs of signals were also observed in the ^{13}C NMR spectra. On the contrary, both the ^{1}H- and ^{13}C NMR spectra of **7** are simple (see Fig. 5b) because the porphyrin macrocycle lacking chiral carbon centres caused the fifth-coordinated complexes as a pair of enantiomers. The much larger upfield shifts of the fifth-coordinated ligand (•) compared to those of the residual benzyl thiol (○) (see arrows in Fig. 5) can be explained by a stronger π-electron ring current in the fully conjugated π-system of the porphyrin moiety. Because the chemical shifts of the residual benzyl thiol protons are consistent with those in the absence of Ga(III) chlorin/porphyrin, this thiol was not the sixth coordinate. The simple spectrum of **7** also shows that the partial sixth ligation with other species (i.e., a water molecule or any anion lacking protons) does not bring the formation of distinguishable complexes as in **1**–**4** on a NMR time-scale. Thus, it was confirmed that the observed inequivalent (1:1.3–1.5) pair of NMR signals of Ga(III) chlorins **1**–**4** reflect diastereomers derived from the fifth-asymmetric axial ligation (α/β-ligation) towards the chiral chlorin π-macrocycle. Such a small difference ($\Delta G = 0.2$ kcal/mol^{-1}) between the diastereomeric complexes is consistent with previous reports[2,3] based on crystallographic analysis of chlorophyll pigments and theoretical expectation (vide supra). Since NOE relation could not be observed between the fifth-ligand and chlorin substituents, it has not been apparent yet which side of the chlorin macrocycles is favoured for axial ligation in this solution system.

In summary, we developed new model compounds useful for investigation of coordination chemistry of natural chlorophylls. The present Ga(III) chlorins **1**–**4**

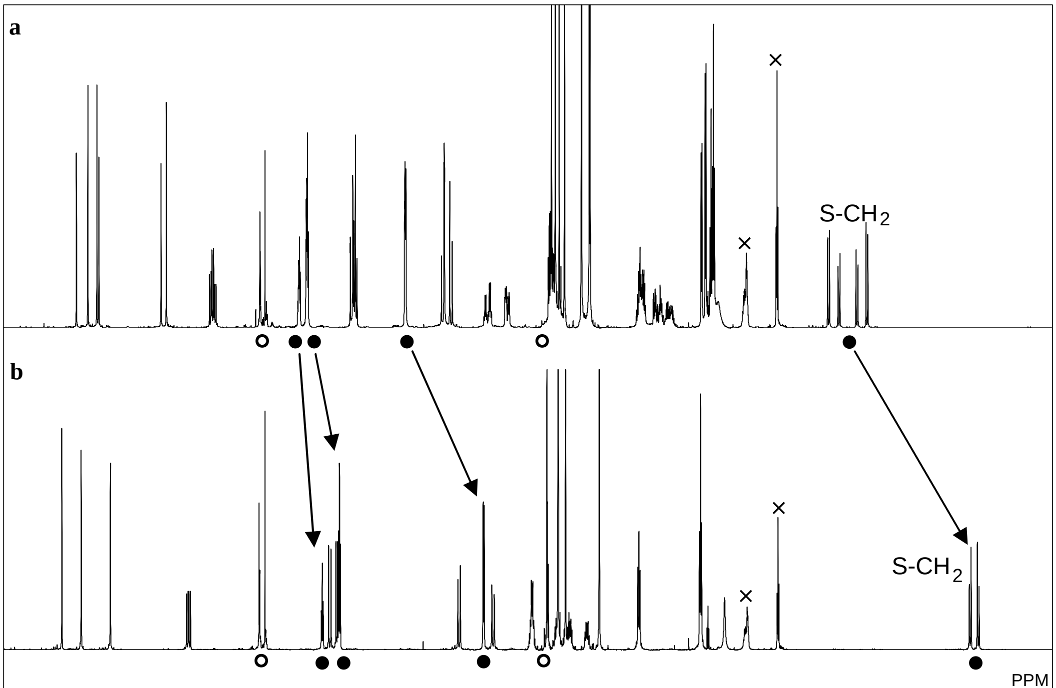


Figure 5. ^{1}H NMR spectra of (a) Ga(III) chlorin **4** and (b) Ga(III) porphyrin **7** in $CDCl_3$. (●) Benzyl thiol moiety as the fifth ligand, (○) residual benzyl thiol, (×) hexane.

showed a pair of NMR signals with different integration ratio in solution, reflecting the energy difference of the diastereomerically controlled fifth-axial ligation. Since the experimental way to investigate the coordination chemistry towards the asymmetric π-faces of chlorophylls had been totally dependent on the X-ray crystal structure analysis, the present axially (α/β)-ligated complexes were discriminated by NMR spectroscopy in solution, providing a much more convenient system for investigation of the asymmetrically ligated chlorophylls. Synthesis of Ga(III) chlorins having other substituents and ligands, and the study of their corresponding In(III) chlorins[22] are in progress.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.11.081.

References and notes

1. Scheer, H. In *Light-Harvesting Antennas in Photosynthesis*; Green, B. R., Parson, W. W., Eds.; Kluwer Academic Publishers: Dordrecht, 2003; pp 29–81.
2. Oba, T.; Tamiaki, H. *Bioorg. Med. Chem.* **2005**, *13*, 5733.
3. Oba, T.; Tamiaki, H. *Photosynth. Res.* **2002**, *74*, 1.
4. Balaban, T. S.; Fromme, P.; Holzwarth, A. R.; Krauss, N.; Prokhorenko, V. I. *Biochim. Biophys. Acta* **2002**, *1556*, 197.
5. Tamiaki, H.; Yagai, S.; Miyatake, T. *Bioorg. Med. Chem.* **1998**, *6*, 2171.
6. van Gammeren, A. J.; Hulsbergen, F. B.; Erkelens, C.; de Groot, H. J. M. *J. Biol. Inorg. Chem.* **2004**, *9*, 109.
7. Tamiaki, H.; Amakawa, M.; Holzwarth, A. R.; Schaffner, K. *Photosynth. Res.* **2002**, *71*, 59.
8. Sanders, J. K. M.; Bampos, N.; Clyde-Watson, Z.; Darling, S. L.; Hawley, J. C.; Kim, H.-J.; Mak, C. C.; Webb, S. J.. In Kadish, K. M., Smith, K. M., Guilard, R., Eds.; The Porphyrin Handbook; Academic Press: San Diego, 2000; Vol. 3, pp 1–48.
9. Harvey, P. D.; Proulx, N.; Martin, G.; Drouin, M.; Nurco, D. J.; Smith, K. M.; Bolze, F.; Gros, C. P.; Guilard, R. *Inorg. Chem.* **2001**, *40*, 4134.
10. Nagata, N.; Kugimiya, S.; Fujiwara, E.; Kobuke, Y. *New J. Chem.* **2003**, *27*, 743.
11. Faure, S.; Stem, C.; Guilard, R.; Harvey, P. D. *J. Am. Chem. Soc.* **2004**, *126*, 1253.
12. Malinowska, E.; Niedziółka, J.; Meyerhoff, M. E. *Anal. Chim. Acta* **2001**, *432*, 67.
13. Nakae, Y.; Fukusaki, E.; Kajiyama, S.; Kobayashi, A.; Nakajima, S.; Sakata, I. *J. Photochem. Photobiol., A Chem.* **2005**, *172*, 55.
14. Litwinski, C.; Tannert, S.; Jesorka, A.; Katterle, M.; Röder, B. *J. Porphyrins Phthalocyanines* **2004**, *8*, 751.
15. Tamiaki, H.; Takeuchi, S.; Tsudzuki, S.; Miyatake, T.; Tanikaga, R. *Tetrahedron* **1998**, *54*, 6699.
16. Kadish, K. M.; Cornillon, J.-L.; Coutsolelos, A.; Guilard, R. *Inorg. Chem.* **1987**, *26*, 4167.
17. Synthesis of compound **1**: to a solution of methyl pyropheophorbide-*a* (3.0 g, 5.5 mmol) in CHCl$_3$ (250 ml) was added GaCl$_3$ (5.4 g, 31 mmol) in MeOH (100 ml) and refluxed for 20 h. After cooling, the reaction mixture was poured into water and extracted with CH$_2$Cl$_2$. The organic phase was concentrated, and the crude product was purified by silica gel chromatography (Et$_2$O–CH$_2$Cl$_2$, 1:9, then MeOH–CH$_2$Cl$_2$, 1:9) to give slow-moving Ga(III)Cl complex (1.42 g, 40%) and fast-moving unreacted free-base chlorin (1.68 g, 56%). The gallium complex was passed through an alumina column deactivated with 6% (w/w) water (MeOH–CH$_2$Cl$_2$, 1:9) for ligand exchange. Recrystallization from CH$_2$Cl$_2$–hexane gave Ga(III)(OH) chlorin **1** (1.28 g) as a green solid. Data for **1**: mp > 300 °C; ^{1}H NMR (600 MHz, CDCl$_3$, 0.01 M, −50 °C): δ (1/1.3) = 9.67/9.56 (1H, s, 10-H), 9.39/9.38 (1H, s, 5-H), 8.63/8.56 (1H, s, 20-H), 7.98 (1H, dd, J = 12, 18 Hz, 3-CH), 6.16–6.20 (2H, m, 3^1-CH$_2$), 5.18, 5.06 (each 1H, d, J = 19 Hz, 13^1-CH$_2$), 4.57/4.52 (1H, br q, J = 7 Hz, 18-H), 4.34 (1H, m, 17-H), 3.68-3.77 (2H, m, 8-CH$_2$), 3.69/3.62 (3H, s, 12-CH$_3$), 3.61/3.59 (3H, s, COOCH$_3$), 3.38/3.37 (3H, s, 2-CH$_3$), 3.26 (3H, s, 7-CH$_3$), 2.56–2.70 (2H, m, 17^1-CH$_2$), 2.18–2.42 (2H, m, 17-CH$_2$), 1.92/1.80 (3H, d, J = 7 Hz, 18-CH$_3$), 1.72/1.68 (3H, t, J = 8 Hz, 8^1-CH$_3$), −5.15/−4.97 (1H, s, OH); UV–vis (CHCl$_3$): $\lambda_{\max}$ 658 (ε, 76,500), 560 (6800), 519 (5000), 425 nm (112,000); IR (KBr pellet) 1734, 1684, 1558, 1543, 1508, 1456, 1211, 989, 928, 804, 745, 706 cm^{-1}; MS (FAB, NBA) m/z 615 [M−OH]$^+$, 632 [M]$^+$, 767 [M−OH+NBA]$^+$: Selected chemical shifts of ^{13}C NMR (150 MHz, CDCl$_3$, 0.03 M, 20 °C): δ (minor/major) = 129.29 (3^2C), 122.74/122.77 (3^1C), 106.19/106.29 (10C), 105.03/105.33 (15C), 99.30/99.14 (5C), 92.96/92.58 (20C), 23.27/23.04 (18C), 19.49/19.44 (8^2C), 17.25 (8^1C).
18. Tamiaki, H.; Watanabe, T.; Miyatake, T. *J. Porphyrins Phthalocyanines* **1999**, *3*, 45.
19. Okamura, T.; Nishikawa, N.; Ueyama, N.; Nakamura, A. *Chem. Lett.* **1998**, 199.
20. Ueno, T.; Nishikawa, N.; Moriyama, S.; Adachi, S.; Lee, K.; Okamura, T.; Ueyama, N.; Nakamura, A. *Inorg. Chem.* **1999**, *38*, 1199.
21. Ligand exchange of **1** with benzyl thiol: to a solution of **1** in CH$_2$Cl$_2$ was added ca. 10 equiv of benzyl thiol, stirred for 30 min at room temperature and then concentrated. The excess thiol was washed out with hexane. The residue was dried in vacuo, resolved in CDCl$_3$ and subjected to measurement. The ^{1}H NMR spectrum clearly showed the completion of ligand exchange to **4** with a small amount of residual benzyl thiol. The sample of Ga(III) porphyrin **7** was similarly prepared from methyl protopyropheophorbide-*a*.
22. The same diastereomeric descrimination was preliminarily observed for several fifth-coordinated In(III) chlorophyll derivatives in their ^{1}H NMR spectra. Similar spectra were recently available in the Supplementary data of Chen, Y.; Zheng, X.; Dobhal, M. P.; Gryshuk, A.; Morgan, J.; Dougherty, T. J.; Oseroff, A.; Pandey, R. K. *J. Med. Chem.* **2005**, *48*, 3692.