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SYNTHESIS OF ISOTOPICALLY LABELED ZINC METHYL BACTERIOPHEOPHORBIDE-D AS A MODEL FOR LIGHT-HARVESTING ANTENNA PIGMENTS

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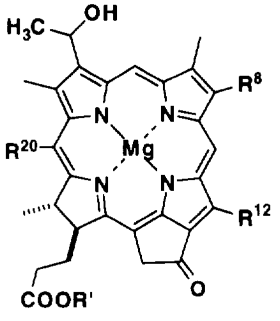
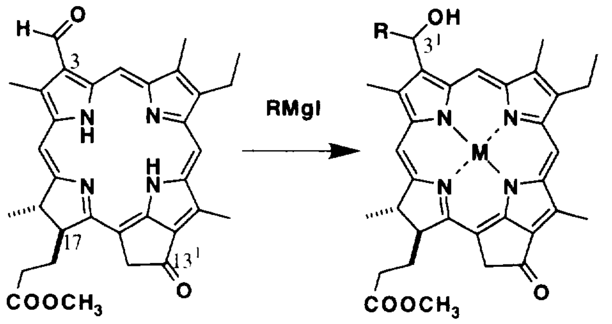
Abstract: Grignard reaction of methyl pyropheophorbide-*d* (**1**) possessing a 3-formyl group with methyl magnesium iodide selectively gave methyl bacteriopheophorbide-*d* (**2**) possessing a 3-(1-hydroxyethyl) group. When $^{13}CH_3MgI$ and CD_3MgI were used as the Grignard reagents, specifically labeled **2** were afforded, of which zinc complexes **3** serve as model compounds for natural bacteriochlorophylls-*d*.

Isotopically labeled compounds are useful for determination of supramolecular structures in biological systems by several techniques including magnetic resonance and vibrational spectroscopies. Chlorophyll derivatives labeled with isotopes (D, ^{13}C, ^{14}C, ^{15}N, ^{18}O) were prepared by biosynthetic[1,2] and synthetic methods[3]. Especially, self-aggregates of uniformly carbon-13 labeled bacteriochlorophyll(=BChl)s-*c* were prepared as models for the main light-harvesting complexes (chlorosomes) of photosynthetic green bacteria and the supramolecular structures were studied by means of ^{13}C-NMR spectroscopy[2]. Site-selectively labeled BChls (> 95% ^{13}C) are necessary for the structural elucidation but have not been synthesized to our knowledge. Here, we report on a convenient synthesis of specifically labeled methyl bacteriopheophorbides-*d* (**2**); the corresponding zinc complexes **3** are good models for natural BChls-*c* and *d*[4].



R^8, R^{12} = alkyl groups
R' = a long chain
R^{20} = CH_3 : BChl-*c*
R^{20} = H : BChl-*d*



1

M = H_2
R = CH_3 : **2a**
R = $^{13}CH_3$: **2b**
R = CD_3 : **2c**

M = Zn
R = CH_3 : **3a**
R = $^{13}CH_3$: **3b**
R = CD_3 : **3c**

To an ice-chilled dry THF solution of methyl pyropheophorbide-*d* (**1**)[5] was added dropwise a diethyl ether solution of methyl magnesium iodide under nitrogen. Visible spectra of the reaction mixture showed that a 695-nm Qy-band characteristic of **1** decreased and concomitantly a new 660-nm band increased. When the ratio of these absorbancies, A_{695} / A_{660} was 1 / 1.2, the addition was stopped and the reaction was quenched with an aqueous solution of saturated ammonium chloride. After separation of the starting material **1** (the first, red fraction, 37%) with the main product (the second, green fraction) by silica gel column chromatography (dichloromethane and methanol as eluents), recrystallization from dichloromethane and hexane gave the desired compound **2a**[6] in a yield of 75% based on consumed **1**. When $^{13}CH_3I$ (99% ^{13}C, Isotec) and CD_3I (99.5% D, Aldrich) were used for preparation of the Grignard reagent, isotopically labeled **2b** and **2c** were afforded as a major isolable product in yields of 79% and 81%, respectively. These specifically labeled compounds were characterized by ^1H-NMR, ^{13}C-NMR and MS spectra[7]. All the synthetic compounds **2** have a new chiral center at the 3^1-position and are diastereomeric mixtures, 3^1-*R* / 3^1-*S* = 1 / 1. Diastereomeric selectivity was not induced by the Grignard reaction because the reaction center at the 3-formyl group was far from the chiral centers, 17- and 18-positions in the molecule. The Grignard reagent site-selectively attacked the 3-formyl group in spite of the presence of 13^1-keto carbonyl group and ester group on the 17-position, indicating that the 3-formyl group is more reactive than the other carbonyl groups in the molecule. Moreover, it is not necessary to protect the chlorin inner NH which is one of the reactive sites in metal-free compound **1**[8].

Compounds **2** were metallated to give zinc complexes **3** quantitatively[4,5]. All the zinc complexes **3a-c** showed the same behaviors in their visible spectra and no isotopic effects were observed. Isotopically labeled **3b** and **3c** were monomeric in dichloromethane (λ_{max} (Q_y-band) = 648 nm) and aggregated to form oligomers in 1% (*v*/*v*) dichloromethane and cyclohexane (λ_{max} (Q_y) = 705 nm) by comparison with a previous work of **3a**[4]. Zinc complexes **3** serve as model systems for natural chlorosomal aggregates. Preparation of self-aggregates of diastereomerically pure **3**[4] and analysis of ^{13}C-NMR and IR spectra of the aggregates in solid states are in progress.

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References and Notes

1. (a) Swanson, K. L.; Smith, K. M. *J. Chem. Soc., Chem. Commun.* **1990**, 1696–1697. (b) Porra, R. J.; Schäfer, W.; Cmiel, E.; Katheder. I.; Scheer, H. *FEBS Lett.* **1993**, 323, 31–34. (c) Käss, H.; Rautter, J.; Bönigk, B.; Höfer, P.; Lubitz, W. *J. Phys. Chem.* **1995**, 99, 436–448. (d) Okazaki, T.; Kajiwara, M. *Chem. Pharm. Bull.* **1995**, 43, 1311–1317.
2. (a) Nozawa, T.; Ohtomo, K.; Suzuki, M.; Nakagawa, H.; Shikama, Y.; Konami, H.; Wang, Z.-Y. *Photosynth. Res.* **1994**, 41, 211–223. (b) Balaban, T. S.; Holzwarth, A. R.; Schaffner, K.; Boender, G.-J.; de Groot, H. J. M. *Biochemistry* **1995**, 34, 15259–15266.
3. (a) Dougherty, R. C.; Strain, H. H.; Katz, J. J. *J. Am. Chem. Soc.* **1965**, 87, 104–109. (b) Fischer, R.; Engel, N.; Henseler, A.; Gossauer, A. *Helv. Chim. Acta* **1994**, 77, 1046–1050.
4. Tamiaki, H.; Takeuchi, S.; Tanikaga, R.; Balaban, S. T.; Holzwarth, A. R.; Schaffner, K. *Chem. Lett.* **1994**, 401–402.
5. Tamiaki, H.; Amakawa, M.; Shimono, Y.; Tanikaga, R.; Holzwarth, A. R.; Schaffner, K. *Photochem. Photobiol.* **1996**, 63, 92–99.
6. Smith, K. M.; Bisset, G. M. F.; Bushell, M. J. *J. Org. Chem.* **1980**, 45, 2218–2224.
7. **2a**: ^1H-NMR ($CDCl_3$) δ 6.39/6.37 (1H, q, J = 7 Hz, 3^1-H), 2.12 (3H, d, J = 7 Hz, 3^1-CH_3); MS (FAB) *m/z* 566 (M^+). **2b**: ^1H-NMR ($CDCl_3$) δ 6.38/6.36 (1H, dq, $^2J_{CH}$ = 3 Hz, $^3J_{HH}$ = 7 Hz, 3^1-H), 2.11 (3H, dd, $^1J_{CH}$ = 127 Hz, $^3J_{HH}$ = 7 Hz, 3^1-CH_3); ^{13}C-NMR ($CDCl_3$) δ 25.73/25.67 (3^1-CH_3); MS (FAB) *m/z* 567 (M^+). **2c**: ^1H-NMR ($CDCl_3$) δ 6.39/6.37 (1H, s, 3^1-H); MS (FAB) *m/z* 569 (M^+).
8. Usually metallation (Ni, Cu, Zn, etc.) is used for protection of the inner NH.

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