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Effects of C8-substituents on spectroscopic and self-aggregation properties of synthetic bacteriochlorophyll-*d* analogues

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Abstract

As a model compound of bacteriochlorophyll(BChl)-*d* which is known as an antenna pigment of green photosynthetic bacteria, methyl bacteriopheophorbide-*d* derivatives having a series of substituents at the 8-position and their zinc complexes were synthesized. Unnatural type analogues were prepared by Wittig reaction of the 8-formyl group, and the 3^1-epimers were separated by reverse-phase HPLC. Absorption spectra of both the synthetic free-base and zinc chlorins in CH_2Cl_2 showed that the C8-substituents conjugating directly with the chlorin ring shifted the Soret and Q_x peaks to longer wavelengths while retaining the position of the Q_y band at around 650 nm. Typically, the observed Q_x peaks in wavelength were situated in the order of ethyl ≈ phenylethyl < vinyl ≈ *cis*-styryl < *trans*-styryl as the C8-substituent reflecting the degree of the conjugation with the chlorin π-system. In 1% (v/v) CH_2Cl_2–cyclohexane, all the epimeric zinc chlorins showed red-shifts of the Q_y bands (≈690–710 nm) and stronger CD peaks in the red-shifted Q_y region, compared to those in CH_2Cl_2. These spectroscopic changes indicated the formation of well-ordered self-aggregates of the synthetic zinc chlorins in non-polar organic solvents similar to BChl-*d* aggregates in a natural antenna system. It was shown that the effect of π-conjugation or sterical hindrance of the C8-substituents does not strongly affect the self-aggregation.

Keywords: Bacteriochlorophyll; Chlorin; Self-aggregation; Photosynthesis antenna

1. Introduction

Chlorosome, an extramembrane antenna of green photosynthetic bacteria, is a unique light-harvesting apparatus. It is known that bacteriochlorophylls(BChls)-*c*, -*d*, and -*e* in chlorosome self-aggregate to form antennas without any assistance from proteins [1–3]. This system is in contrast to those of innermembrane antennas which consist of pigment–protein complexes [3,4]. So far, several models of this supramolecular structure have been proposed based on many experiments using isolated natural chlorophylls [1,3,5–11] or synthetic model compounds [1,12–17]. Since self-aggregation of these BChls is caused by the intermolecular interaction among the 3^1-hydroxyl, central metal, and 13-carbonyl moieties, together with π–π interaction of chlorin macrocycles, many model compounds with different substituents along the Q_y axis (N21–N23 in Fig. 1a) have been designed and synthesized, and their characteristics of self-aggregation properties have been investigated [17–19]. On the contrary, such effects of the substituents along the Q_x axis (N22–N24 in Fig. 1a) have been investigated less [20–22], mainly due to their poor availability.

Natural chlorosomal chlorophylls consist of several homologs with different alkyl substituents. For example, BChl-*d* is composed of homologs having different alkyl groups at the 8- and 12-positions (R^8 and R^{12}) as shown in Fig. 1a. Although several homologs of BChl-*d* have been separated and their self-aggregation properties have been reported [11], no report, to our best knowledge, is available investigating the effect of the substituents at the 8-position systematically. In order to explore a synthetic route to model compounds of BChl-*d* with an alkyl group at the 8-position, we earlier reported the synthesis of methyl bacteriopheophorbide(MBPhe)-*d* possessing 8-propyl group (R^8 = Pr in Fig. 1b) [23]. The key step was Grignard reaction of the 8-formyl group, and the metal-free MBPhe-*d* was obtained as a 3^1-epimeric mixture. As an alternative way using the Wittig reaction, here we designed and synthesized the series of BChl-*d* analogues with various C8-substituents shown in Fig. 1b. The resulting metal-free MBPhes-*d* were monomeric species in CH_2Cl_2 and their visible spectral characteristics were elucidated. Furthermore, self-aggregation properties of epimerically pure zinc

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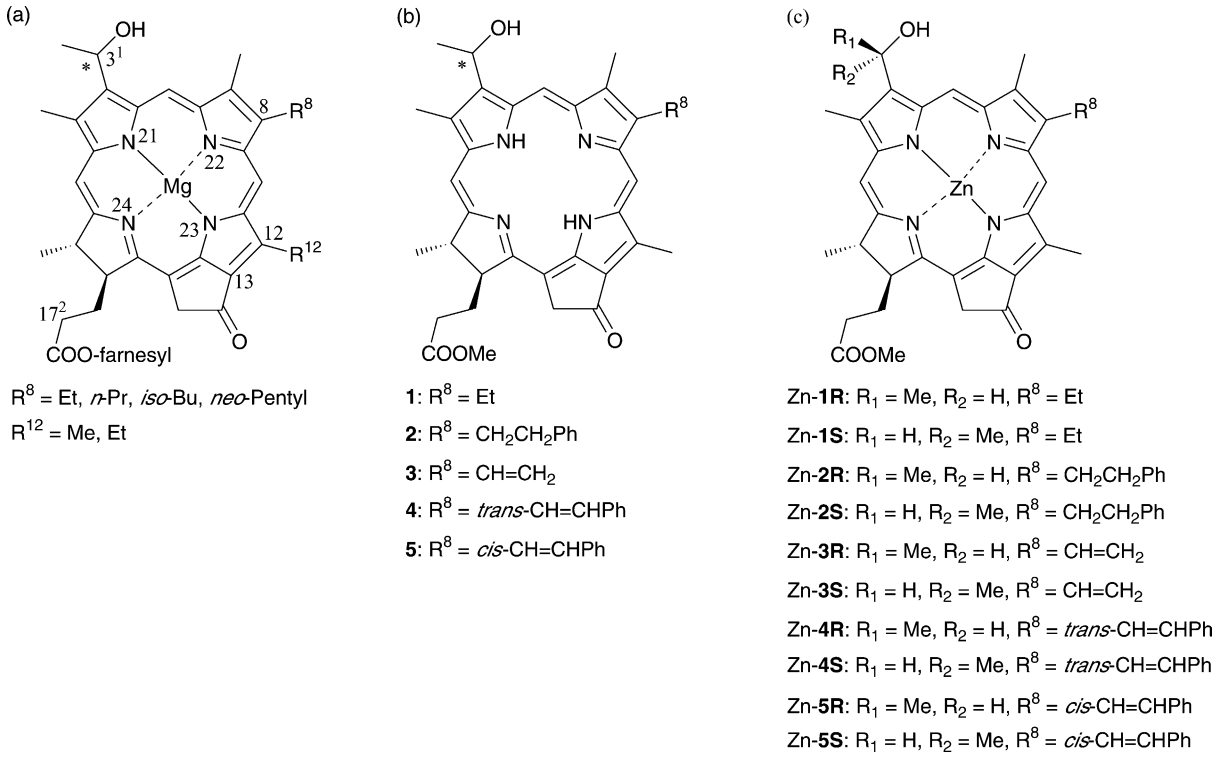


Fig. 1. Molecular structures of (a) naturally occurring magnesium–chlorin, bacteriochlorophylls-*d* (BChls-*d*), (b) their synthetic analogues, methyl bacteriopheophorbides-*d* (MBPhes-*d*), and (c) 3^1-epimeric zinc complexes (Zn–MBPhes-*d*).

complexes of MBPhes-*d* (=Zn–MBPhes-*d*) were also examined in a non-polar organic solvent. It was demonstrated that the substituents at the 8-position do not strongly affect the supramolecular structure of the self-aggregation along the Q_y axis, although they do cause significant shifts of the Soret and Q_x peaks in their monomeric forms.

2. Experimental

2.1. General

^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer. Coupling constants are given in Hertz, and all chemical shifts are reported relative to the residual solvent peak: $\delta_H = 7.26$ ppm ($CHCl_3$). Visible absorption and CD spectra were measured on a Hitachi U-3500 spectrophotometer and a JASCO J-720W spectropolarimeter, respectively. FAB-MS spectra were recorded on a JEOL HX-100 spectrophotometer. HPLC was carried out with a packed ODS column (Gelpack GL-OP100, Hitachi Chemical Co., 6.0 mmϕ × 150 mm or Cosmosil 5C18-ARII, 10 mmϕ × 250 mm). Methyl 3-acetyl-3-devinyl-pyropheophorbide-*a* (**6**) was prepared from methyl pyropheophorbide-*a* according to the reported procedure [24]. Zinc-metallation of free base chlorin was done according to the reported procedure [24,25]. Methyl 8-ethyl-12-methyl-bacteriopheophorbide-*d* (**1**) and the 3^1-epimerically pure zinc complexes (Zn–**1R/S**) were prepared as previously reported [26]. Other reagents were employed as purchased without further purification. All synthetic procedures were done in the dark.

2.2. Synthesis of metal-free MBPhes-d

2.2.1. Synthesis of methyl 3-acetyl-3-devinyl-7,8-cis-dihydroxy-pyropheophorbide-a (7)

To a solution of **6** (940 mg, 1.57 mmol) in CH_2Cl_2 (150 ml) was added OsO_4 (1.0 g, 3.8 mmol) and pyridine (5 ml), and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with MeOH (100 ml) and bubbled with H_2S for 20 min, then filtered through Celite, and the filtrate was concentrated. The crude product was purified by chromatography on silica gel (MeOH–CH_2Cl_2, 2:98) to give 7,8-*cis*-diol **7** (758 mg, 81%) as a 4:3 diastereomeric mixture. λ_{max} (nm) (CH_2Cl_2) 746 (rel. 57%), 534 (27), 361 (100); δ_H ($CDCl_3$, major/minor = 4/3) 9.17/9.12, 8.74/8.73, 8.35/8.31 (each 1H, s, 5-, 10-, 20-H), 4.98, 4.86 (each 1H, d, J = 20, 13^1-CH_2), 4.25–4.27 (1H, m, 18-H), 4.09–4.11 (1H, m, 17-H), 3.64/3.61, 3.48, 3.41/3.35, 3.17/3.15 (each 3H, s, 2-, 3^1-, 12-CH_3, $COOCH_3$), 2.45–2.60, 2.22–2.34 (each 2H, m, 17-CH_2CH_2), 2.34/2.30 (3H, s, 7-CH_3), 2.16–2.19 (2H, m, 8-CH_2), 1.75/1.69 (3H, d, J = 7, 18-CH_3), 1.42/1.25 (3H, t, J = 7, 8^1-CH_3), 0.84/0.71, −0.68/−0.80 (each 1H, s, NH); *m/z* (FAB) 598 (M^+).

*2.2.2. Synthesis of methyl 3-acetyl-3-devinyl-8-deethyl-8-vinyl-pyropheophorbide-a (**8**)*

To a solution of diol **7** (150 mg, 0.25 mmol) in CH_2Cl_2 (5 ml) and benzene (5 ml) was added *p*-TsOH·H_2O (70 mg, 0.37 mmol), and the mixture was stirred for 4 h at room temperature. Then 10 ml of benzene was added, gradually heated, and refluxed for 30 min. After cooling, the reaction mixture was poured into 5% aqueous HCl, which was extracted with $CHCl_3$. The organic phase was washed with 5% aqueous $KHSO_4$, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (Et_2O–hexane, 5:95) to give 8-vinylchlorin **8** [27] (40 mg, 28%) as a black solid. A mixture of mono-dehydrated intermediates was collected with 3% MeOH–CH_2Cl_2 as an eluent. The mono-alcoholic intermediates were further dehydrated under the conditions described above to give additional **8**. The total yield was 44%. λ_{max} (nm) (CH_2Cl_2) 683 (rel. 51%), 621 (11), 520 (16), 426 (100), 384 (60); δ_H ($CDCl_3$) 10.01, 9.76, 8.79 (each 1H, s, 5-, 10-, 20-H), 7.95 (1H, dd, $J = 12$, 18, 8-CH), 6.18 (1H, dd, $J = 1$, 18, 8^1-CH *trans* to 8-CH), 6.30 (1H, dd, $J = 1$, 12, 8^1-CH *cis* to 8-CH), 5.29, 5.17 (each 1H, d, $J = 20$, 13^1-CH_2), 4.55–4.57 (1H, m, 18-H), 4.35–4.38 (1H, m, 17-H), 3.70, 3.67, 3.62, 3.41, 3.30 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.55–2.76, 2.26–2.38 (each 2H, m, 17-CH_2CH_2), 1.85 (3H, d, $J = 7$, 18-CH_3), −2.10 (1H, s, NH [another NH was too broad to be observed]); *m*/*z* (FAB) 562 (M^+).

*2.2.3. Synthesis of methyl 3-acetyl-3-devinyl-8-deethyl-8-formyl-pyropheophorbide-a (**9**)*

According to reported procedures [24], 8-vinylchlorin **8** (86 mg, 0.015 mmol) was oxidized by OsO_4 (ca. 20 mg), $NaIO_4$ (160 mg, 0.75 mmol), AcOH (0.5 ml) in water (6 ml) and THF (30 ml). The crude product was recrystallized from CH_2Cl_2–hexane to give 8-formylchlorin **9** (71 mg, 83%) as a black solid. λ_{max} (nm) (CH_2Cl_2) 677 (rel. 33%), 614 (13), 535 (21), 446 (100); δ_H ($CDCl_3$) 10.75 (1H, s, CHO), 9.88, 9.80, 9.71 (each 1H, s, 5-, 10-, 20-H), 5.28, 5.10 (each 1H, d, $J = 20$, 13^1-CH_2), 4.56–4.62 (1H, m, 18-H), 4.36–4.39 (1H, m, 17-H), 3.69, 3.67, 3.44, 3.28, 3.27 (each 3H, s, 2-, 7-, 3^1-, 12-CH_3, $COOCH_3$), 2.61–2.64, 2.33–2.38 (each 2H, m, 17-CH_2CH_2), 1.94 (3H, d, $J = 7$, 18-CH_3), −0.87, −2.43 (each 1H, s, NH); *m*/*z* (FAB) 564 (M^+).

*2.2.4. Synthesis of methyl 3-acetyl-3-devinyl-8-deethyl-8-styryl-pyropheophorbide-a (**10** and **11**)*

Wittig reaction of 8-formylchlorin **9** (40 mg, 0.071 mmol) with benzyltriphenylphosphonium chloride (70 mg, 0.18 mmol) in CH_2Cl_2 (20 ml) and 0.15 M aqueous NaOH (5 ml) was performed as reported [28]. The crude product was purified by chromatography on silica gel (Et_2O–CH_2Cl_2, 1:19) to give *cis*-isomer **10** (9.5 mg, 21%) as a fast moving band ($R_f = 0.3$) and *trans*-isomer **11** (13 mg, 29%) as a slow moving band ($R_f = 0.2$). Both isomers were black solids. **10**: λ_{max} (nm) (CH_2Cl_2) 683 (rel. 45%), 623 (7), 519 (12), 427 (100), 388 (59); δ_H ($CDCl_3$) 9.99, 9.60, 8.80 (each 1H, s, 5-, 10-, 20-H), 7.63 (1H, d, $J = 12$, 8-CH), 7.33 (2H, d, $J = 7$, ph-2,6-H), 7.30 (1H, d, $J = 12$, 8^1-CH), 6.96 (2H, t, $J = 7$, ph-3,5-H), 6.93 (1H, t, $J = 7$, ph-4-H), 5.30, 5.15 (each 1H, d, $J = 20$, 13^1-CH_2), 4.56 (1H, dq, $J = 2$, 7, 18-H), 4.34–4.36 (1H, m, 17-H), 3.66, 3.62, 3.52, 3.28, 3.08 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.56–2.75, 2.24–2.34 (each 2H, m, 17-CH_2CH_2), 1.83 (3H, d, $J = 7$, 18-CH_3), −1.99 (1H, s, NH [another NH was too broad to be observed]); *m*/*z* (FAB) 638 (M^+). **11**: λ_{max} (nm) (CH_2Cl_2) 683 (rel. 34%), 625 (6), 521 (13), 434 (100), 389 (47); δ_H ($CDCl_3$) 10.05, 9.72, 8.79 (each 1H, s, 5-, 10-, 20-H), 8.30 (1H, d, $J = 17$, 8-CH), 7.87 (2H, d, $J = 7$, ph-2,6-H), 7.56 (2H, t, $J = 7$, ph-3,5-H), 7.50 (1H, d, $J = 17$, 8^1-CH), 7.42 (1H, t, $J = 7$, ph-4-H), 5.33, 5.17 (each 1H, d, $J = 20$, 13^1-CH_2), 4.57 (1H, dq, $J = 2$, 7, 18-H), 4.38 (1H, dt, $J = 7$, 2, 17-H), 3.67, 3.67, 3.63, 3.46, 3.30 (each 3H, s, 2-, 3^1-, 7-, 12-CH_3, $COOCH_3$), 2.58–2.79, 2.30–2.37 (each 2H, m, 17-CH_2CH_2), 1.86 (3H, d, $J = 7$, 18-CH_3), −0.15, −2.06 (each 1H, s, NH); *m*/*z* (FAB) 638 (M^+).

*2.2.5. Synthesis of methyl 8-cis-styryl-12-methyl-bacteriopheophorbide-d (**5**)*

To a stirred solution of *cis*-isomer **10** (1.8 mg, 2.8 μmol) in CH_2Cl_2 was added dropwise a solution of $NaBH_4$ (2.4 mg, 0.063 mmol) in MeOH (3 ml), and the reaction was monitored by TLC. After being stirred for a few minutes at room temperature (disappearance of the TLC spot of **10**), the reaction mixture was quenched by the addition of water, which was extracted with CH_2Cl_2. The organic phase was washed with water, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (MeOH–CH_2Cl_2, 1:99) to give 3-(1-hydroxyethyl)-chlorin **5** (1.3 mg, 72%) as a 3^1-epimeric mixture (1:1). λ_{max} (nm) (CH_2Cl_2) 662 (rel. 40%), 605 (9), 541 (7), 510 (11), 419 (100); δ_H ($CDCl_3$) 9.71/9.69, 9.51, 8.55 (each 1H, s, 5-, 10-, 20-H), 7.62 (1H, d, $J = 12$, 8-CH), 7.34 (2H, d, $J = 7$, ph-2,6-H), 7.30 (1H, d, $J = 12$, 8^1-CH), 6.97 (2H, t, $J = 7$, ph-3,5-H), 6.94 (1H, t, $J = 7$, ph-4-H), 6.45 (1H, m, 3-CH), 5.24, 5.08 (each 1H, d, $J = 20$, 13^1-CH_2), 4.46–4.50 (1H, m, 18-H), 4.27–4.30 (1H, m, 17-H), 3.61, 3.49, 3.43, 3.05 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.48–2.75, 2.24–2.36 (each 2H, m, 17-CH_2CH_2), 2.16 (3H, d, $J = 7$, 3^1-CH_3), 1.79/1.80 (3H, d, $J = 7$, 18-CH_3), −1.79 (1H, s, NH [another NH was too broad to be observed]); *m*/*z* (FAB) 640 (M^+).

*2.2.6. Synthesis of methyl 8-(2-phenylethyl)-12-methyl-bacteriopheophorbide-d (**2**)*

To a solution of *cis*-olefin **5** (2.0 mg, 3.1 μmol) in 1,4-dioxane (2 ml) was added PtO_2 (3.3 mg, 0.015 mmol), and the suspension was stirred for 2 days at 50 °C under an atmosphere of hydrogen. The mixture was filtered to remove insoluble catalyst and diluted with CH_2Cl_2. The filtrate was washed with water, dried over Na_2SO_4, filtered, and concentrated. The crude product was

purified by HPLC (GL-OP100, MeOH, 2.0 ml min^{-1}) followed by recrystallization from CH_2Cl_2–hexane to give 8-(2-phenylethyl)chlorin **2** (1.5 mg, 75%) as a 3^1-epimeric mixture (1:1). λ_{max} (nm) (CH_2Cl_2) 660 (rel. 46%), 604 (11), 536 (12), 504 (14), 411 (100); δ_H ($CDCl_3$) 9.67/9.65, 9.25, 8.57/8.52 (each 1H, s, 5-, 10-, 20-H), 7.17–7.26 (5H, m, ph-H), 6.42–6.45 (1H, m, 3-CH), 5.23, 5.09 (each 1H, d, $J = 20$, 13^1-CH_2), 4.46–4.48 (1H, m, 18-H), 4.27–4.29 (1H, m, 17-H), 3.95 (2H, t, $J = 7$, 8-CH_2), 3.62/3.61, 3.58, 3.42/3.41, 3.08 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 3.37 (2H, t, $J = 7$, 8^1-CH_2), 2.55–2.67, 2.27–2.32 (each 2H, m, 17-CH_2CH_2), 2.14 (3H, d, $J = 7$, 3^1-CH_3), 1.80/1.77 (3H, d, $J = 7$, 18-CH_3), −2.25 (1H, s, NH [another NH was too broad to be observed]); *m/z* (FAB) 642 (M^+).

2.2.7. Synthesis of methyl 8-vinyl-12-methyl-bacteriopheophorbide-d (3)

Reduction of 3-acetyl group of **8** was carried out as described for the preparation of **5** to give **3** in 82% yield as a 3^1-epimeric mixture (1:1). λ_{max} (nm) (CH_2Cl_2) 660 (rel. 37%), 605 (6), 541 (5), 509 (8), 418 (100); δ_H ($CDCl_3$) 9.76/9.73, 9.57/9.56, 8.53/8.51 (each 1H, s, 5-, 10-, 20-H), 7.90 (1H, dd, $J = 12$, 18, 8-CH), 6.44 (1H, m, 3-CH), 6.13 (1H, d, $J = 18$, 8^1-CH *trans* to 8-CH), 5.99 (1H, d, $J = 12$, 8^1-CH *cis* to 8-CH), 5.20, 5.05 (each 1H, d, $J = 20$, 13^1-CH_2), 4.47–4.49 (1H, m, 18-H), 4.25–4.28 (1H, m, 17-H), 3.63, 3.62, 3.43/3.42, 3.38 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.54–2.68, 2.23–2.34 (each 2H, m, 17-CH_2CH_2), 2.14 (3H, d, $J = 7$, 3^1-CH_3), 1.81/1.77 (3H, d, $J = 7$, 18-CH_3), −1.93/−1.95 (1H, s, NH [another NH was too broad to be observed]); *m/z* (FAB) 564 (M^+).

2.2.8. Synthesis of methyl 8-trans-styryl-12-methyl-bacteriopheophorbide-d (4)

Reduction of 3-acetyl group of *trans*-isomer **11** was carried out as described for the preparation of **5** to give 3-(1-hydroxyethyl)-8-(*trans*-styryl)chlorin **4** in 77% yield as a 3^1-epimeric mixture (1:1). λ_{max} (nm) (CH_2Cl_2) 663 (rel. 36%), 607 (7), 512 (11), 423 (100); δ_H ($CDCl_3$) 9.84/9.72, 9.51/9.50, 8.53/8.52 (each 1H, s, 5-, 10-, 20-H), 8.22 (1H, d, $J = 17$, 8-CH), 7.83 (2H, d, $J = 7$, ph-2,6-H), 7.54 (2H, t, $J = 7$, ph-3,5-H), 7.49 (1H, d, $J = 17$, 8^1-CH), 7.42 (1H, t, $J = 7$, ph-4-H), 6.41 (1H, q, $J = 7$, 3-CH), 5.24, 5.06 (each 1H, d, $J = 20$, 13^1-CH_2), 4.47 (1H, dq, $J = 2$, 7, 18-H), 4.24–4.26 (1H, m, 17-H), 3.63/3.62, 3.54, 3.42/3.41, 3.39 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.61–2.73, 2.25–2.31 (each 2H, m, 17-CH_2CH_2), 2.15 (3H, d, $J = 7$, 3^1-CH_3), 1.79/1.80 (3H, d, $J = 7$, 18-CH_3), −1.91/−1.92 (1H, s, NH [another NH was too broad to be observed]); *m/z* (FAB) 640 (M^+).

2.3. Synthesis of 3^1-epimerically pure Zn–MBPhes-d

MBPhes-*d* (**2**–**5**) were zinc-metallated, and the crude products were purified by chromatography on silica gel (MeOH–CH_2Cl_2, 1∼3:99∼97) followed by HPLC (MeOH) to give zinc chlorins Zn–**1**–**5** as an epimeric mixture ($3^1R/3^1S = 1/1$). Each epimer was further separated by HPLC (MeOH–H_2O, 4∼5:1).

Zn–**2**: t_R 8.5 min (Gelpack, MeOH, 1.5 ml min^{-1}); λ_{max} (nm) (CH_2Cl_2) 647 (rel. 70%), 600 (14), 557 (9), 515 (8), 423 (100); *m/z* (FAB) 704 (M^+). Retention times (Cosmosil, MeOH–H_2O, 5:1, 2 ml min^{-1}) were 51 and 55 min for Zn–**2R** and Zn–**2S**, respectively.

Zn–**3**: t_R 9 min (Cosmosil, MeOH, 2 ml min^{-1}); λ_{max} (nm) (CH_2Cl_2) 647 (rel. 76%), 603 (13), 560 (7), 517 (8), 429 (100); *m/z* (FAB) 626 (M^+). Retention times (Cosmosil, MeOH–H_2O, 4:1, 2 ml min^{-1}) were 58 and 63 min for Zn–**3R** and Zn–**3S**, respectively.

Zn–**4**: t_R 10 min (Cosmosil, MeOH, 2 ml min^{-1}); λ_{max} (nm) (CH_2Cl_2) 650 (rel. 67%), 603 (13), 563 (6), 523 (8), 439 (100); *m/z* (FAB) 702 (M^+). Retention times (Cosmosil, MeOH–H_2O, 8.5:1.5, 2 ml min^{-1}) were 28 and 30 min for Zn–**4R** and Zn–**4S**, respectively.

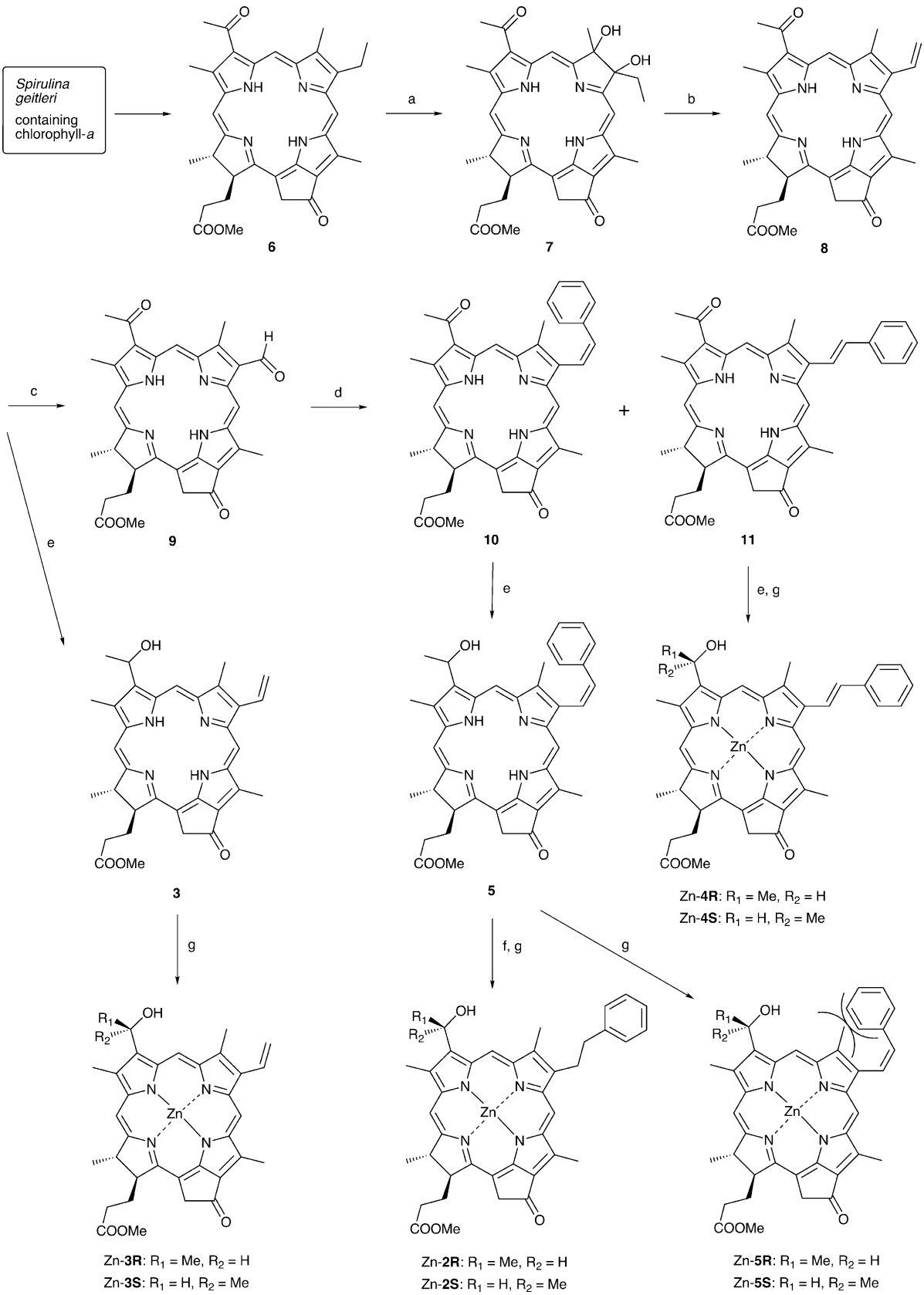
Zn–**5**: t_R 10 min (Cosmosil, MeOH, 2 ml min^{-1}); λ_{max} (nm) (CH_2Cl_2) 649 (rel. 77%), 603 (13), 561 (6), 518 (7), 429 (100); *m/z* (FAB) 702 (M^+). Retention times (Cosmosil, MeOH–H_2O, 4:1, 2 ml min^{-1}) were 36 and 39 min for Zn–**5R** and Zn–**5S**, respectively.

3. Results and discussion

3.1. Design and synthesis of BChl-d analogues

Metal-free chlorins (MBPhes-*d*) synthesized in this study are shown in Fig. 1b. These compounds were designed to examine the effect of the steric hindrance or the π-conjugation at the 8-position. The structural characteristics for each of the chlorins are as follows: compound **1** with the 8-ethyl group has the same peripheral substituents as a naturally occurring BChl-*d*, except the alkyl chain on the 17^2 ester (farnesyl → methyl ester). Compound **2** has a 2-phenylethyl group at the 8-position, whose phenyl π-ring does not directly conjugate with the chlorin π-system but is bulky at the ethyl terminal. On the other hand, the vinyl group of **3** can conjugate with the chlorin π-macrocycle. The styryl groups of **4** and **5** should be more conjugatable and/or bulky. Moreover, the *cis*-isomer **5** should disturb the π-conjugation due to steric repulsion between phenyl and chlorin rings as expected.

The synthesis of each of these model compounds is outlined in Scheme 1. Methyl 3-acetyl-3-devinyl-pyropheophorbide-*a* (**6**), which was prepared by modification of chlorophyll-*a* extracted from *Spirulina geitleri* (a cyanobacterium) according to the reported procedures [24], was used as a starting material. Addition of **6** with OsO_4 in the presence of pyridine and cleavage of the resulting cyclic ester by H_2S [24,29] gave 7,8-*cis*-diol **7** in 81% yield as a ca. 3:4 diastereomeric mixture. The resulting *cis*-diol **7** was doubly dehydrated by treatment of *p*-TsOH in benzene [24] to give the desired 3-acetyl-8-vinyl-chlorin **8** in 32% yield. This low yield is ascribable to the electron-withdrawing 3-acetyl



Scheme 1. Synthesis of (Zn–)MBPhes-*d*, (Zn–)**2**–**5**. Reagents and conditions: (a) (i) OsO_4, pyridine, CH_2Cl_2; (ii) H_2S(g), CH_2Cl_2–MeOH, 81%; (b) *p*-TsOH·H_2O, CH_2Cl_2–benzene, 44%; (c) OsO_4, $NaIO_4$, aq.AcOH–THF, 83%; (d) $PhCH_2PPh_3Cl$, aq.NaOH–CH_2Cl_2, **10**: 21%, **11**: 29%; (e) $NaBH_4$, CH_2Cl_2–MeOH, **3**: 82%, **4**: 77%, **5**: 72% (f) PtO_2, H_2(g), 1,4-dioxane, 54%; (g) (i) $Zn(OAc)_2{\cdot}2H_2O$, CH_2Cl_2–MeOH, (ii) HPLC separation.

substituent. The 8-vinyl group of **8** was oxidatively cleaved by OsO_4 and $NaIO_4$ [24,25] to give 3-acetyl-8-formyl chlorin **9** (83%) [23], which was subjected to the following Wittig reaction.

The reaction between 8-formylchlorin **9** and benzyltriphenylphosphonium chloride was performed in a way similar to that previously reported [28]. The product was separated by column chromatography into *cis*-isomer **10** (21%) and *trans*-isomer **11** (29%). The moderate isolated yield (50%) is the result of the incomplete separation of the mixture by silica gel chromatography due to their similar R_f values. The first fraction (R_f = 0.3 in 5% Et_2O–CH_2Cl_2) was assigned to *cis*-isomer **10** based on the ^{1}H-NMR spectrum, which showed characteristic signals at δ 7.63 and 7.30 with the coupling constants of $^3J(8^1H\text{-}8^2H)$ = 12 Hz. The NMR spectrum of the second fraction (R_f = 0.2) showed a pair of doublets at δ 8.30 and 7.50 (J = 17 Hz), which was assigned to be *trans*-isomer **11**. The 3-acetyl group of each separated isomer was then reduced by $NaBH_4$ into the 3-(1-hydroxyethyl) group to give metal-free **4** and **5** in 77% and 72% yield, respectively.

The 3-(1-hydroxyethyl)-8-(*cis*-styryl)chlorin **5** was hydrogenated with H_2 gas in the presence of excess PtO_2 to afford 8-(2-phenylethyl)chlorin **2** (75%). As expected [30], the *trans*-isomer **4** did not react with H_2 under the same conditions, and alternative hydrogenation of *cis*-isomer **5** using Pd–C as a catalyst also failed. Selective hydrogenation of the *cis*/*trans* mixture (**5**/**4**) using PtO_2 was successful in giving **2** and **4**, but tedious HPLC purification was required for the separation between the desired **2** and the unreacted *trans*-isomer **4**.

The 3-acetyl group of **8** was reduced by $NaBH_4$ to give 3-(1-hydroxyethyl)-8-vinyl-chlorin **3**. From the ^{1}H-NMR spectral analysis, all the synthetic 3-(1-hydroxyethyl)-chlorins **2**-**5** were 1:1 3^1-epimeric mixtures, because reduction of the 3-acetyl group by $NaBH_4$ proceeded non-stereoselectively. After insertion of the central zinc by standard procedures [24,25], the resulting 3^1-epimers of Zn–BPhes-*d* were separated by reverse-phase HPLC to give the epimerically pure compounds. The first and second fractions were assigned to 3^1R and 3^1S epimers, respectively, because it has been shown that all of the 3^1-epimers examined so far including Zn–**1** are eluted in the order of 3^1R and 3^1S under such HPLC conditions [5,10,20,22,26,31].

Table 1
Absorption maxima (λ_{max} (nm)) of methyl bacteriopheophorbides-*d* **1**–**5**[a]

Compound	Soret	Q_x	Q_y
1	409	504, 535	660
2	411	504, 536	660
3	418	509, 541	660
4	423	512[b]	663
5	419	510, 541	662

[a] 3^1R/S (=1/1) epimeric mixtures were measured in CH_2Cl_2 at ca. 10 μM.
[b] A broad peak was observed at ca. 550 nm.

3.2. Visible absorption spectra of metal-free MBPhes-*d* **1**–**5**

Absorption spectra of **1**-**5** were measured in CH_2Cl_2 and the results are summarized in Table 1. Q_y maxima of these compounds having alkyl/alkenyl groups at the 8-position are almost the same. While the Q_y bands changed little, the absorption maxima of Soret and Q_x peaks showed clearer variations reflecting the character of the C8-substituents. Fig. 2 shows the absorption spectra of **1**, **3**, and **4** at the Q_x region. All the absorption maxima (>300 nm) of **2** are essentially the same as those of **1**, indicating that the bulkiness of the phenylethyl group without further π-conjugation does not affect the spectrum of free-base chlorin in CH_2Cl_2. Contrary to compound **2**, the Soret and Q_x peaks of **3**–**5** moved to longer wavelengths than those of the natural type model **1**, apparently due to the prolonged conjugation at the 8-position. It should be noted that the introduction of substituents at the 3-position induced a similar shift of

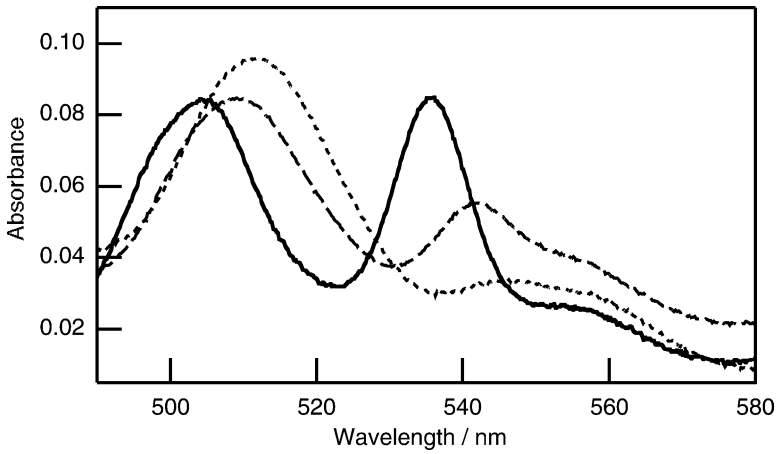


Fig. 2. Absorption spectra of metal-free chlorins (MBPhes-*d*) **1**: solid line, **3**: dashed line, and **4**: dotted line at the Q_x region in CH_2Cl_2 at ca. 10 μM.

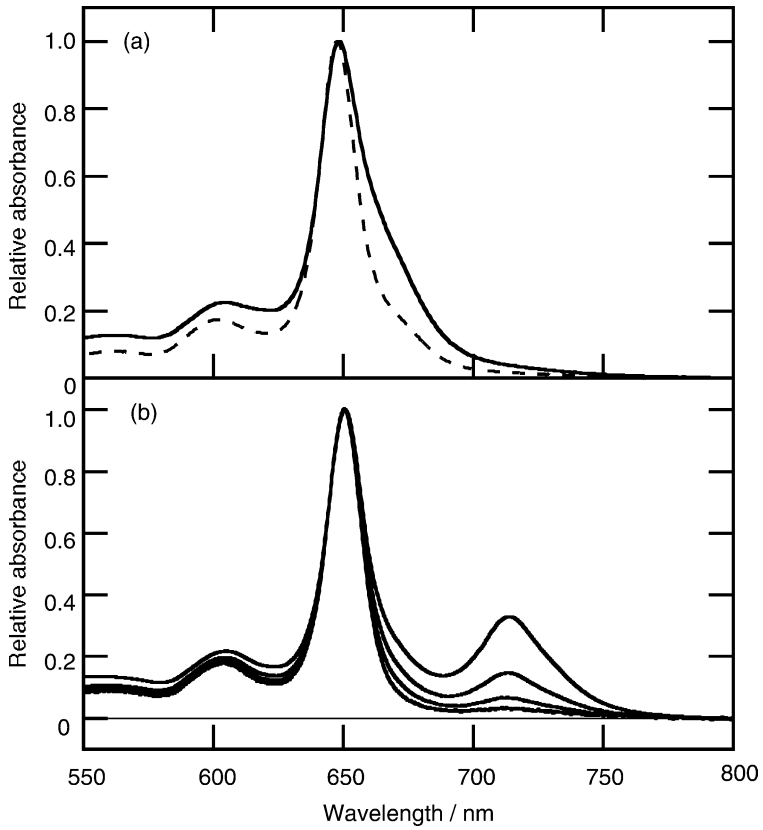


Fig. 3. Absorption spectra of epimeric zinc chlorins (Zn–MBPhes-*d*) measured in CH_2Cl_2. (a) Solid line and dashed lines represent the spectra of Zn–**3R** and Zn–**3S** measured at ca. 10 μM, respectively. (b) Zn–**4R** with concentrations of ca. 5, 10, 15, and 20 μM. All spectra were normalized at their major Q_y peaks.

Q_y maximum due to the prolonged conjugation [28]. The Soret/Q_x peak positions of *trans*-styryl **4** (423/512 nm) are more red-shifted than those of vinyl **3** (418/509 nm), while *cis*-styryl **5** (419/510 nm) gives almost the same maxima as **3**. Because the phenyl ring of *cis*-isomer **5** is less conjugated with the chlorin ring due to the steric repulsion (vide supra), the fewer observed shifts in **3** → **5** can be explained by the reduced π-conjugation.

3.3. Visible absorption spectra of epimeric Zn–MBPhes-d Zn–1–5R/S in CH_2Cl_2

Absorption spectra of epimerically pure zinc chlorins Zn–**1–5R/S** were measured in CH_2Cl_2. Compared to the sharp Q_y absorption bands for epimeric Zn–**1R/S** and Zn–**2R/S**, Zn–**3R** showed a relatively broad Q_y band as can be seen in Fig. 3a and gave a shoulder on the red side of the monomeric Q_y peak. The shoulder is ascribed to a dimer of Zn–**3R**, based on the previous report [26] that the chloro/fluoro substituents at the 20-position induce a similar dimer formation even in CH_2Cl_2 solution. Zn–**4R** in CH_2Cl_2 also showed both major monomeric (650 nm) and a minor additional peak at the longer wavelength. As shown in Fig. 3b, the minor peak is positioned at 714 nm. The value indicates the formation of a higher order aggregate rather than a dimer, compared with reported data [13]. It is interesting to note that Zn–**4R** in CH_2Cl_2 shows concentration dependent spectra without apparent formation of the dimer band. Such phenomena were not observed for the corresponding Zn–**3S** and Zn–**4S**.

Major absorption maxima of epimeric zinc chlorins Zn–**1**–**5R/S** are summarized in Table 2. In CH_2Cl_2 solution, little difference was observed between 3^1R and 3^1S epimers as can be seen in Fig. 4a. The absorption maxima of the Soret and Q_x peaks of each monomer are red-shifted in the

Table 2
Absorption maxima (λ_{max}/nm) of epimeric zinc chlorins **1**–**5**

Compound	CH_2Cl_2			1% CH_2Cl_2–cyclohexane
	Soret	Q_x	Q_y	Q_y
Zn–**1R**	422	513	648	705
Zn–**1S**	422	513	648	693
Zn–**2R**	423	515	647	703
Zn–**2S**	423	515	647	701
Zn–**3R**	430	519	648, 670 (sh)	704
Zn–**3S**	430	519	648	700
Zn–**4R**	440	524	650, 714	713
Zn–**4S**	440	524	650	696
Zn–**5R**	430	519	649	703
Zn–**5S**	430	519	649	703

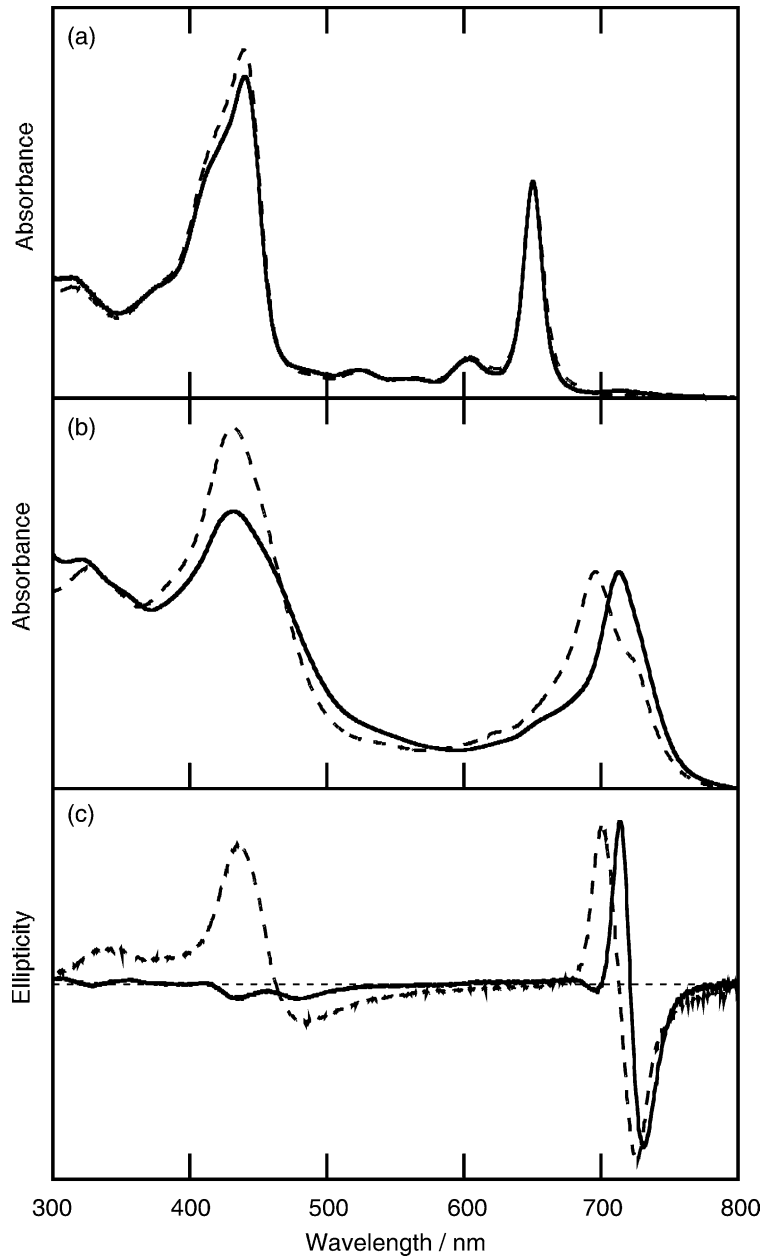


Fig. 4. Absorption spectra of epimeric Zn–**4** in (a) CH_2Cl_2 and (b) 1% CH_2Cl_2–cyclohexane, and CD spectra of (c) epimeric Zn–**4** in 1% CH_2Cl_2–cyclohexane. Solid and dashed lines represent the spectra of Zn–**4R** and Zn–**4S**, respectively. All the spectra are normalized at the Q_y peak.

order of Zn–**1** (Soret, Q_x: 422, 513 nm) ≈ Zn–**2** (423, 515) < Zn–**3** (430, 519) ≈ Zn–**5** (430, 519) < Zn–**4** (440, 524), while their Q_y peaks are almost the same at around 648 nm. This tendency is consistent with those of the free-base MBPhes-*d*, reflecting the effect of the C8-substituents.

*3.4. Self-aggregation properties of Zn–MBPhes-d Zn–**1**–**5R/S** in non-polar organic solvent*

In non-polar organic solvent such as 1% CH_2Cl_2–cyclohexane, zinc chlorins Zn–**1–5R/S** self-aggregated to form oligomers, which was confirmed by the large red-shift and broadening of Q_y peak [13,26] as represented by the spectra of Zn–**4R/S** in Fig. 4b. The observed S-shape CD peaks at the region of red-shifted Q_y band also suggest the formation of the well-ordered aggregates. Similar spectral changes were observed for Zn–**1**–**3**/**5**. It should be pointed out that 3^1R epimers of Zn–MBPhes-*d* tend to form aggregates with more red-shifted Q_y peaks than those of 3^1S epimers (except in Zn–**5R/S**), although the differences are dependent on the C8-substituents. Among the examined compounds, self-aggregates of Zn–**4R/S** with *trans*-styryl group showed the largest diastereomeric control in the Q_y band. The red-shift in the Q_y peaks accompanied by the

formation of aggregates is 63 and 46 nm for Zn–**4R** and Zn–**4S**, respectively, and the difference of the Q_y peaks in the aggregation form is 343 cm^{-1}. Since the Q_y peak of Zn–**4R** (713 nm) is located at a more red-shifted region (ca. 10 nm) than the other Zn–**R** aggregates, the *trans*-styryl group might support formation of the tighter self-aggregates through any additional intermolecular interaction. However, the Q_y peak shifts caused by the formation of the aggregates and the Q_y peak differences between each epimer in their oligomeric form are comparable to those of the reported synthetic zinc chlorins [30]. Therefore, it is concluded that the C8-substituents do not affect the self-aggregation of synthetic Zn–MBPhes-*d* to any great degree.

4. Conclusion

A series of bacteriochlorophyll-*d* analogues (Zn–) MBPhes-*d* having various substituents at the 8-position were synthesized, and their visible spectra dependent upon the C8-substituent were examined in the monomeric and self-aggregated species. The prolonged conjugation at the 8-position caused red-shifts of the Soret and Q_x peaks while almost retaining the position of Q_y bands in their monomeric forms. On the other hand, all Zn–MBPhes-*d* showed a similar red-shift of the Q_y bands in non-polar organic solvent compared to those of the monomeric forms in CH_2Cl_2, supporting the formation of self-aggregates along the Q_y axis. It was found that self-aggregates of the 3^1R epimers showed more red-shifted Q_y peaks compared to the corresponding 3^1S epimeric aggregates. Thus, it was concluded that the C8-substituents had less effect on the supramolecular structures of the self-aggregates along the Q_y axis, which were greatly influenced by the stereochemistry of the 1-hydroxyethyl group at the 3-position.

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