Journal of Photochemistry and Photobiology A: Chemistry 207 (2009) 115–125

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Synthesis of galactosylated zinc bacteriochlorophyll-*d* analogs and their self-aggregation in an aqueous methanol solution

Hitoshi Tamiaki*, Ai Shinkai, Yumiko Kataoka

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan

ARTICLE INFO

Article history:
Available online 20 February 2009

Keywords:
Chlorophyll–glycoside conjugate
Chlorosome
Light-harvesting antenna
Photosynthesis
Self-assembly

ABSTRACT

Chlorophyll derivatives possessing a galactosyl moiety at the 17-substituent were prepared by condensation of a carboxy group in the 17-substituent of chlorin chromophores with an amino group on the galactosides having four unprotected hydroxy moieties. Synthetic zinc 3-hydroxymethyl-13^1-oxochlorins covalently linked with the galactosyl group through a spacer of various lengths self-aggregated in an aqueous methanol solution to give a slipped cofacial dimer and large oligomers with a red-shifted Q_y absorption band. The resulting oligomers were similar to J-aggregates of bacteriochlorophyll-*d* in main light-harvesting antennas of green photosynthetic bacteria. Electronic absorption spectral analyses of the hydrophilic chlorophyll derivatives in the aqueous solution indicated that their self-aggregation was dependent on the spacer; the shorter afforded their dimers predominantly and the longer disturbed the formation of chlorosomal large oligomers.

1. Introduction

Most porphyrinoids are hydrophobic due to the extended π-systems of their cyclic tetrapyrroles. To increase their affinity to an aqueous solution, various hydrophilic substituents have been introduced in their molecules, which are useful for medicinal application. For example, hydrophilic (bacterio)chlorins are promising for photodynamic therapy (PDT) of any cancers because of their high affinity to water as well as their intense absorption of lights from red to near infrared regions which penetrate to deep areas of tissues [1]. To prepare such hydrophilic chlorins, many functional groups have been examined, i.e., polyethers, polyamines, amino acids, cations, strong acids, sugars, and so on [1–9].

In contrast, some naturally occurring (bacterio)chlorophylls are embodied inside proteins and lipid assemblies to give water-soluble apparatus in photosynthetic organisms [10–15]. Typically, main light-harvesting antennas of green photosynthetic bacteria (chlorosomes) are one such system, where many chlorophyll molecules including bacteriochlorophyll-*d* self-aggregate inside a lipid monolayer containing proteins (see left of Fig. 1) to form large J-aggregates absorbing >700-nm light [16]. In green sulfur bacteria, glycolipids are main lipid components of the chlorosomal envelop and monogalactosyldiglycerides (MGDG) were found in vivo [17]. In vitro self-aggregates of the composite chlorophylls in an aqueous MGDG assembly were reported to mimic the light-harvesting and energy-migrating functions as well as supramolecular structures of natural chlorosomes [18–21]. Here we report chlorosomal self-aggregates of synthetic model compound **1** in an aqueous octyl β-D-galactoside micelle (see middle of Fig. 1). Moreover, the two components were covalently linked with various spacers and the resulting galactosylated zinc 3-hydroxymethyl-13^1-oxochlorins **11**, **15** and **16** (see right of Fig. 1) were examined for self-aggregation in an aqueous 1% (v/v) methanol solution by electronic absorption spectroscopy.

2. Experimental

2.1. General

Visible absorption spectra were measured in air-saturated solvents at room temperature on a Hitachi U-3500 spectrophotometer. ^{1}H NMR spectra were recorded at 293 K on a Bruker AC-300 (300 MHz), JEOL AL-400 (400 MHz) or ECA-600 (600 MHz) spectrometer; as an internal reference, $CDCl_3$ (δ = 7.26 ppm) or tetramethylsilane (δ = 0 ppm) was used. Liquid chromatography–mass spectroscopy (LC–MS) was performed with Shimadzu LCMS-2010EV: electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) were used. Fast atom bombardment-mass spectroscopy (FAB-MS) data were measured by a JEOL Gcmate II spectrophotometer, and *m*-nitrobenzyl alcohol and glycerol were used as matrixes. Flash column chromatography (FCC) was performed with silica gel (Merck, Kieselgel 60). High performance liquid chromatography (HPLC) was done with a Shimadzu LC-10ADvp pump and SPD-M10Avp photodiode-array

* Corresponding author. Fax: +81 77 561 2659.
E-mail address: tamiaki@se.ritsumei.ac.jp (H. Tamiaki).

1010-6030/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2009.01.008

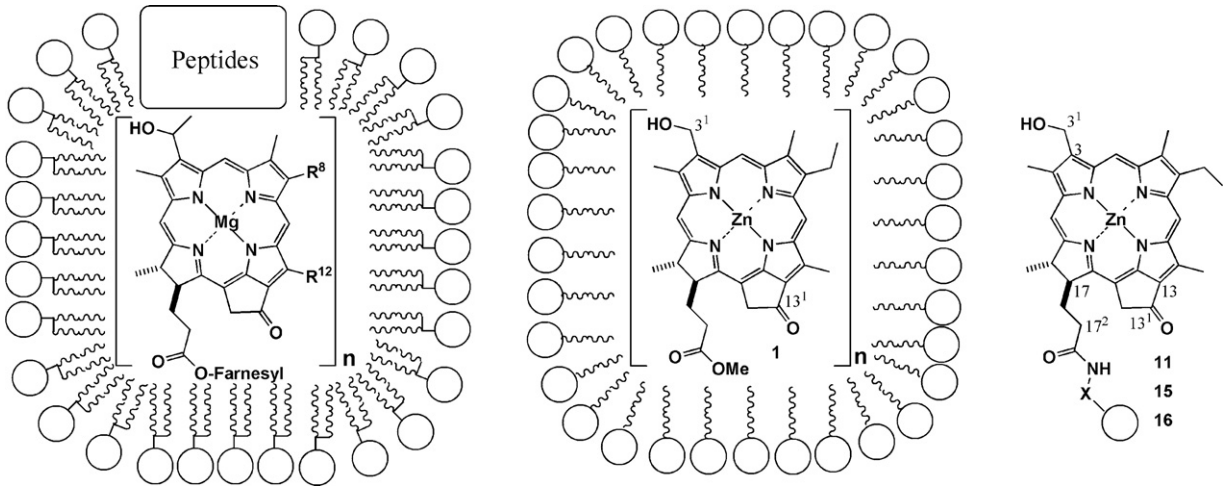


Fig. 1. Schematic drawings of natural chlorosome containing bacteriochlorophyll-*d* (left), artificial chlorosome containing synthetic zinc chlorophyll derivative **1** (middle) and molecular structure of galactosylated zinc chlorophyll derivatives **11**, **15** and **16** (right, **X** is a series of covalent linkers). Open circles represent hydrophilic (galactosyl) moieties and wave lines indicate hydrophobic aliphatic chains.

detector. Galactosides on thin-layer chromatography (TLC) were detected after being stained by 5% H_2SO_4–EtOH solution.

Pyropheophorbide-*d* was prepared by the reported procedures [22]. Methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* and its zinc complex **1** were synthesized according to the literature [23]. Methyl 12-aminododecanoate hydrochloride was prepared by the reported procedures [24] and its data are available from Ref. [25].

2.2. Synthesis of galactose derivatives

2.2.1. 1-O-(2-bromoethyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**2a**)

Boron trifluoride etherate (BF_3Et_2O, 2.6 ml) was added to a CH_2Cl_2 solution (30 ml) of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (β-D-galactose pentaacetate, 5.0 g) and 2-bromoethanol (2.80 ml). The mixture was stirred at room temperature under N_2 overnight, washed with aqueous 4% $NaHCO_3$ and aqueous saturated NaCl, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by FCC (23–25% AcOEt–hexane) to give **2a** (3.94 g, 68%) (79% in Ref. [26] and 67% in Ref. [27]); ^{1}H NMR ($CDCl_3$) δ = 5.35 (1H, dd, J = 1, 3.5 Hz, 4-H), 5.18 (1H, dd, J = 8, 10.5 Hz, 2-H), 4.99 (1H, dd, J = 3.5, 10.5 Hz, 3-H), 4.51 (1H, d, J = 8 Hz, 1-H), 4.13, 4.12 (each 1H, dd, J = 7, 10 Hz, 5-CH_2), 3.89 (1H, dt, J = 1, 7 Hz, 5-H), 3.78, 3.48 (1H, dt, J = 10, 6 Hz, 1-OCH_2), 3.43 (2H, t, J = 6 Hz, CH_2Br), 2.11, 2.05, 2.02, 1.95 (each 3H, s, CH_3 ×4).

2.2.2. 1-O-(2-azidoethyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**3a**)

A mixture of **2a** (3.94 g) and NaN_3 (0.9 g) in distilled *N*,*N*-dimethyl formamide (DMF, 13.5 ml) was stirred at 70 °C under Ar for 2 h. After addition of AcOEt, the mixture was washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by FCC (50% AcOEt–hexane) to give **3a** (3.59 g, 99%) (81% in Ref. [26], see also Refs. [28,29]); ^{1}H NMR ($CDCl_3$) δ = 5.36 (1H, dd, J = 1, 3 Hz, 4-H), 5.20 (1H, dd, J = 10.5, 8 Hz, 2-H), 4.99 (1H, dd, J = 3, 10.5 Hz, 3-H), 4.53 (1H, d, J = 8 Hz, 1-H), 4.15, 4.13 (each 1H, dd, J = 7, 11 Hz, 5-CH_2), 3.89 (1H, dt, J = 1, 7 Hz, 5-H), 3.75, 3.48 (1H, dt, J = 10, 6 Hz, 1-OCH_2), 3.29 (2H, t, J = 6 Hz, CH_2N_3), 2.11, 2.03, 2.03, 1.95 (each 3H, s, CH_3 ×4).

2.2.3. 1-O-(2-azidoethyl)-β-D-galactopyranose (**4a**)

Acetate **3a** (212 mg) and 28% MeONa–MeOH (110 μl) were dissolved in MeOH (10 ml) at room temperature under N_2. After stirring for 2.5 h, the mixture was neutralized by Amberlite IR-120 (plus) resin, filtered and concentrated. The residue was purified by FCC (10% MeOH–$CHCl_3$) to give **4a** (124 mg, 98%) (89% in Ref. [26] and 99% in Ref. [30]); ^{1}H NMR (CD_3OD) δ = 4.26 (1H, d, J = 8 Hz, 1-H), 4.03 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.71 (1H, d, J = 2 Hz, 4-H), 3.62–3.57 (2H, m, 1-OCH, 2-H), 3.41–3.32 (6H, m, 3-, 5-H, 5-CH_2, CH_2N_3).

2.2.4. 1-O-(2-aminoethyl)-β-D-galactopyranose (**5a**)

Azide **4a** (124 mg) and PtO_2 (100 mg) in MeOH (100 ml) were stirred at room temperature under H_2 for 2 h. The mixture was filtered and concentrated to give **5a** (108 mg, 97%) (90% in Ref. [26], see also Refs. [29,30]); ^{1}H NMR (CD_3OD) δ = 4.27 (1H, d, J = 7 Hz, 1-H), 4.00 (1H, dt, J = 10, 5 Hz, 1-OCH), 3.80 (1H, d, J = 2 Hz, 4-H), 3.75 (2H, m, 1-OCH, 2-H), 3.57–3.45 (4H, m, 3-, 5-H, 5-CH_2), 3.05 (2H, t, J = 5 Hz, CH_2N).

2.2.5. 1-O-(6-bromohexyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**2b**)

Similar to the synthesis of **2a**, reaction of pentaacetate (2.0 g) with 6-bromo-1-hexanol (0.9 ml) in BF_3Et_2O (0.6 ml) and CH_2Cl_2 (12 ml) for 7 h gave **2b** (1.33 g, 51%); ^{1}H NMR ($CDCl_3$) δ = 5.15 (1H, dd, J = 1, 3.5 Hz, 4-H), 4.94 (1H, dd, J = 8, 10.5 Hz, 2-H), 4.81 (1H, dd, J = 3.5, 10.5 Hz, 3-H), 4.29 (1H, d, J = 8 Hz, 1-H), 3.93, 3.92 (each 1H, dd, J = 7, 10 Hz, 5-CH_2), 3.75 (1H, dt, J = 1, 7 Hz, 5-H), 3.67, 3.28 (1H, dt, J = 10, 6 Hz, 1-OCH_2), 3.20 (2H, t, J = 7 Hz, CH_2Br), 1.92, 1.82, 1.81, 1.74 (each 3H, s, CH_3 ×4), 1.63 (2H, m, CH_2CBr), 1.37 (2H, m, 1-$OCCH_2$), 1.19 (4H, m, 1-$OC_2CH_2CH_2$).

2.2.6. 1-O-(6-azidohexyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**3b**)

Similar to the synthesis of **3a**, reaction of **2b** (1.33 g) with NaN_3 (300 mg) in DMF (9 ml) for 2.5 h gave **3b** (1.17 g, 95%); ^{1}H NMR ($CDCl_3$) δ = 5.28 (1H, dd, J = 1, 3 Hz, 4-H), 5.09 (1H, dd, J = 10.5, 8 Hz, 2-H), 4.92 (1H, dd, J = 3, 10.5 Hz, 3-H), 4.38 (1H, d, J = 8 Hz, 1-H), 4.06, 4.04 (each 1H, dd, J = 7, 11 Hz, 5-CH_2), 3.80 (2H, m, 1-OCH, 5-H), 3.39 (1H, dt, J = 10, 6 Hz, 1-OCH), 3.18 (2H, t, J = 7 Hz, CH_2N_3), 2.05, 1.96, 1.95, 1.89 (each 3H, s, CH_3 ×4), 1.50 (4H, m, 1-$OCCH_2$, CH_2CN_3), 1.29 (4H, m, 1-$OC_2CH_2CH_2$).

2.2.7. 1-O-(6-azidohexyl)-β-D-galactopyranose (**4b**)

Similar to the synthesis of **4a**, deprotection of **3b** (867 mg) with 28% MeONa–MeOH (1.35 ml) in MeOH (100 ml) for 1.5 h gave **4b** (470 mg, 84%) (see Refs. [31,32]); ^{1}H NMR (CD_3OD) δ = 4.15 (1H, d, J = 7 Hz, 1-H), 3.84 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.78 (1H, d,

J = 2 Hz, 4-H), 3.68 (1H, dd, J = 2, 7 Hz, 2-H), 3.49 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.46–3.41 (4H, m, 3-, 5-H, 5-CH_2), 3.22 (2H, t, J = 7 Hz, CH_2N_3), 1.59–1.50 (4H, m, 1-$OCCH_2$, CH_2CN_3), 1.36–1.30 (4H, m, 1-$OC_2CH_2CH_2$).

*2.2.8. 1-O-(6-aminohexyl)-β-D-galactopyranose (**5b**)*

Similar to the synthesis of **5a**, hydrogenation of **4b** (308 mg) on PtO_2 (70 mg) in MeOH (50 ml) for 2.5 h gave **5b** (269 mg, 97%) (see Ref. [33]); ^{1}H NMR (CD_3OD) δ = 4.12 (1H, d, J = 7 Hz, 1-H), 3.81 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.74 (1H, d, J = 2 Hz, 4-H), 3.64 (1H, dd, J = 2, 7 Hz, 2-H), 3.46 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.43–3.37 (4H, m, 3-, 5-H, 5-CH_2), 2.56 (2H, t, J = 7 Hz, CH_2N), 1.57–1.53 (2H, m, 1-$OCCH_2$), 1.42–1.37 (2H, m, CH_2CN), 1.37–1.25 (4H, m, 1-$OC_2CH_2CH_2$).

*2.2.9. 1-O-(10-bromodecyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**2c**)*

Similar to the synthesis of **2a**, reaction of pentaacetate (1.5 g) with 10-bromo-1-decanol (0.65 ml) in BF_3Et_2O (0.45 ml) and CH_2Cl_2 (10 ml) for 8 h gave **2c** (735 mg, 39%) (see Ref. [34]); ^{1}H NMR ($CDCl_3$) δ = 5.37 (1H, dd, J = 1, 3.5 Hz, 4-H), 5.18 (1H, dd, J = 8, 10.5 Hz, 2-H), 5.00 (1H, dd, J = 3.5,10.5 Hz, 3-H), 4.44 (1H, d, J = 8 Hz, 1-H), 4.19–4.07 (3H, m, 5-H, 5-CH_2), 3.88, 3.46 (1H, dt, J = 10, 7 Hz, 1-OCH_2), 3.40 (2H, t, J = 7 Hz, CH_2Br), 2.14, 2.04, 2.04, 1.97 (each 3H, s, CH_3 ×4), 1.63–1.26 (16H, m, 1-$OC(CH_2)_8$).

*2.2.10. 1-O-(10-azidodecyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**3c**)*

Similar to the synthesis of **3a**, reaction of **2c** (735 mg) with NaN_3 (152 mg) in DMF (7 ml) for 2.5 h gave **3c** (631 mg, 92%) (99% in Ref. [34]); ^{1}H NMR ($CDCl_3$) δ = 5.37 (1H, dd, J = 1, 3.5 Hz, 4-H), 5.18 (1H, dd, J = 8, 10 Hz, 2-H), 5.00 (1H, dd, J = 3.5, 10 Hz, 3-H), 4.44 (1H, d, J = 8 Hz, 1-H), 4.19–4.10 (3H, m, 5-H, 5-CH_2), 3.87, 3.45 (1H, dt, J = 10, 7 Hz, 1-OCH_2), 3.24 (2H, t, J = 7 Hz, CH_2N_3), 2.09, 2.02, 2.02, 1.96 (each 3H, s, CH_3 ×4), 1.61–1.50 (4H, m, 1-$OCCH_2$, CH_2N_3), 1.40–1.23 (12H, m, 1-$OC_2(CH_2)_6$).

*2.2.11. 1-O-(10-azidodecyl)-β-D-galactopyranose (**4c**)*

Similar to the synthesis of **4a**, deprotection of **3c** (285 mg) with 28% MeONa–MeOH (0.3 ml) in MeOH (70 ml) for 2 h gave **4c** (185 mg, 95%); ^{1}H NMR (CD_3OD) δ = 4.20 (1H, d, J = 7 Hz, 1-H), 3.89 (1H, dt, J = 10, 8 Hz, 1-OCH), 3.83 (1H, d, J = 2 Hz, 4-H), 3.73 (1H, dd, J = 2, 7 Hz, 2-H), 3.54 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.50–3.46 (4H, m, 3-, 5-H, 5-CH_2), 3.27 (2H, t, J = 7 Hz, CH_2N_3), 1.63–1.56 (4H, m, 1-$OCCH_2$, CH_2CN_3), 1.40–1.20 (12H, m, 1-$OC_2(CH_2)_6$).

*2.2.12. 1-O-(10-aminodecyl)-β-D-galactopyranose (**5c**)*

Similar to the synthesis of **5a**, hydrogenation of **4c** (185 mg) on PtO_2 (72.4 mg) in MeOH (50 ml) for 2 h gave **5c** (170 mg, 99%); ^{1}H NMR (CD_3OD) δ = 4.20 (1H, d, J = 7 Hz, 1-H), 3.89 (1H, dt, J = 10, 8 Hz, 1-OCH), 3.83 (1H, d, J = 2 Hz, 4-H), 3.73 (1H, dd, J = 2, 7 Hz, 2-H), 3.54 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.50–3.46 (4H, m, 3-, 5-H, 5-CH_2), 3.27 (2H, t, J = 7 Hz, CH_2N), 1.63–1.56 (4H, m, 1-$OCCH_2$, CH_2CN), 1.40–1.20 (12H, m, 1-$OC_2(CH_2)_6$).

*2.2.13. 1-O-(12-bromododecyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**2d**)*

Similar to the synthesis of **2a**, reaction of pentaacetate (1.5 g) with 12-bromo-1-dodecanol (162 mg) in BF_3Et_2O (0.45 ml) and CH_2Cl_2 (15 ml) for 7.5 h gave **2d** (233 mg, 64%); ^{1}H NMR ($CDCl_3$) δ = 5.36 (1H, dd, J = 1, 3.5 Hz, 4-H), 5.18 (1H, dd, J = 8, 10.5 Hz, 2-H), 4.99 (1H, dd, J = 3.5, 10.5 Hz, 3-H), 4.43 (1H, d, J = 8 Hz, 1-H), 4.19–4.08 (3H, m, 5-H, 5-CH_2), 3.87 3.45 (1H, dt, J = 10, 7 Hz, 1-OCH_2), 3.39 (2H, t, J = 7 Hz, CH_2Br), 2.13, 2.03, 2.03, 1.96 (each 3H, s, CH_3 ×4), 1.60–1.20 (20H, m, 1-$OC(CH_2)_{10}$).

*2.2.14. 1-O-(12-azidododecyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose (**3d**)*

Similar to the synthesis of **3a**, reaction of **2d** (233 mg) with NaN_3 (38.5 mg) in DMF (6 ml) for 2 h gave **3d** (196 mg, 90%); ^{1}H NMR ($CDCl_3$) δ = 5.37 (1H, dd, J = 1, 3 Hz, 4-H), 5.19 (1H, dd, J = 8, 10.5 Hz, 2-H), 5.00 (1H, dd, J = 3, 10.5 Hz, 3-H), 4.43 (1H, d, J = 8 Hz, 1-H), 4.17–4.08 (3H, m, 5-H, 5-CH_2), 3.87, 3.45 (1H, dt, J = 10, 7 Hz, 1-OCH_2), 3.24 (2H, t, J = 7 Hz, CH_2N_3), 2.13, 2.03, 2.03, 1.97 (each 3H, s, CH_3 ×4), 1.62–1.50 (4H, m, 1-$OCCH_2$, CH_2CN_3), 1.40–1.20 (16H, m, 1-$OC_2(CH_2)_8$).

*2.2.15. 1-O-(12-azidododecyl)-β-D-galactopyranose (**4d**)*

Similar to the synthesis of **4a**, deprotection of **3d** (190 mg) with 28% MeONa–MeOH (0.20 ml) in MeOH (50 ml) for 2 h gave **4d** (128 mg, 97%); ^{1}H NMR (CD_3OD) δ = 4.13 (1H, d, J = 7 Hz, 1-H), 3.87 (1H, dt, J = 10, 7 Hz, 1-OCH), 3.71 (1H, d, J = 2 Hz, 4-H), 3.60 (2H, m, 1-OCH, 2-H), 3.40–3.30 (6H, m, 3-, 5-H, 5-CH_2, CH_2N_3), 1.60–1.45 (4H, m, 1-$OCCH_2$, CH_2CN_3), 1.40–1.20 (16H, m, 1-$OC_2(CH_2)_8$).

*2.2.16. 1-O-(12-aminododecyl)-β-D-galactopyranose (**5d**)*

Similar to the synthesis of **5a**, hydrogenation of **4d** (125 mg) on PtO_2 (35 mg) in MeOH (50 ml) for 2 h gave **5d** (116 mg, 99%); ^{1}H NMR (CD_3OD) δ = 4.20 (1H, d, J = 7.5 Hz, 1-H), 3.89, 3.54 (1H, dt, J = 10, 7 Hz, 1-OCH_2), 3.85 (1H, d, J = 2, 4-H), 3.74 (1H, dd, J = 2, 7.5 Hz, 2-H), 3.51–3.45 (4H, m, 3-, 5-H, 5-CH_2), 2.66 (2H, t, J = 8 Hz, CH_2N), 1.60–1.30 (20H, m, 1-$OC(CH_2)_{10}$).

*2.2.17. 1-O-(2-aminoethyl)-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose p-toluenesulfonic acid (**6**)*

Azide **3a** (200 mg) and *p*-toluenesulfonic acid monohydrate (*p*TsOH·H_2O, 52 mg) were dissolved in EtOH (7 ml). Lindlar catalyst (160 mg) was added and the mixture was hydrogenated for 1 h. After another addition of Lindlar catalyst (120 mg), the mixture was further hydrogenated for 1 h, then the catalyst was removed by filtration and the residue was evaporated to dryness. The residue was passed on FCC (10% MeOH–$CHCl_3$). The crude product was dissolved in MeOH and filtered, then the filtrate was concentrated to give **6** (95% in Ref. [28] and see also Ref. [35]).

2.3. Synthesis of chlorophyll derivatives

*2.3.1. Synthesis of 3-devinyl-3-hydroxymethyl-pyropheophorbide-a (**12**)*

A solution of methyl 3-devinyl-3-hydroxymethyl-pyropheophorbide-*a* (142 mg) in concentrated HCl (20 ml) was stirred for 2 h. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The aqueous phase was further extracted with $CHCl_3$ and the combined organic phases were washed with 4% $KHSO_4$ and water, dried over Na_2SO_4 and evaporated to dryness. The residue was recrystallized from CH_2Cl_2–hexane to give **12** (137 mg, 99%); vis (MeOH) λ_{max} = 660 (relative intensity, 0.46), 604 (0.09), 536 (0.09), 504 (0.10), 407 (1.0), 319 nm (0.26); ^{1}H NMR (5% CD_3OD–$CDCl_3$) δ = 9.42, 9.42, 8.53 (each 1H, s, 5-, 10-, 20-H), 5.80 (2H, s, 3-CH_2), 5.19, 5.02 (each 1H, d, J = 20 Hz, 13^1-CH_2), 4.42 (1H, q, J = 8 Hz, 18-H), 4.18 (1H, m, 17-H), 3.63 (2H, q, J = 8 Hz, 8-CH_2), 3.57, 3.36, 3.21 (each 3H, s, 2-, 7-, 12-CH_3), 2.31–2.23, 2.00–1.97 (each 2H, m, 17-CH_2CH_2), 1.74 (3H, d, J = 8 Hz, 18-CH_3), 1.64 (3H, t, J = 8 Hz, 8^1-CH_3); MS (ESI) found: m/z 537. Calcd for $C_{32}H_{33}N_4O_4$: $[M-H]^-$, 537.

*2.3.2. Galactosylated chlorophyll with ethylene spacer **10a***

Acid **12** (60 mg) and amine **5a** (91 mg) were dissolved in distilled DMF (12 ml). 1-Hydroxy-benzotriazole (HOBt, 69 mg) and 1-ethyl-3-(*N*,*N*-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 83 mg) were added to the solution at room temperature

under Ar. After stirring for 19 h, $CHCl_3$ was added to the reaction mixture and the mixed solution was washed with aqueous 4% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated to dryness. The residue was purified with FCC (12–13% MeOH–$CHCl_3$) and successively by recrystallization from MeOH/$CHCl_3$/CCl_4–hexane to give **10a** (46.3 mg, 56%) (see Ref. [26]); vis (MeOH) λ_{max} = 660 (rel., 0.50), 604 (0.09), 536 (0.10), 505 (0.10), 407 (1.0), 319 nm (0.25); ^{1}H NMR (40% CD_3OD–$CDCl_3$) δ = 9.14, 9.13, 8.30 (each 1H, s, 5-, 10-, 20-H), 5.53, 5.50 (each 1H, d, J = 13 Hz 3-CH_2), 5.02, 4.80 (each 1H, d, J = 20 Hz, 13^1-CH_2), 4.24 (1H, q, J = 7 Hz, 18-H), 4.01 (1H, d, J = 9 Hz, 17-H), 3.61 (1H, d, J = 8 Hz, 1′-H), 3.40–2.90 (12H, m, 1′-OCH_2CH_2, 2′-, 3′-, 4′-, 5′-H, 5′-, 8-CH_2), 3.31, 3.11, 2.94 (each 3H, s, 2-, 7-, 12-CH_3), 2.40–2.05 (4H, m, 17-CH_2CH_2), 1.53 (3H, d, J = 7 Hz, 18-CH_3), 1.39 (3H, t, J = 8 Hz, 8^1-CH_3); MS (ESI) found: m/z 742. Calcd for $C_{40}H_{48}N_5O_9$: $[M-H]^-$, 742.

2.3.3. Galactosylated zinc chlorophyll with ethylene spacer ***11a***

Free base **10a** was dissolved in $CHCl_3$ and a small amount of MeOH, to which was added a MeOH solution saturated with Zn(OAc)2H_2O. After stirring for 1 h, the reaction mixture was washed with aqueous 4% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated to dryness. The residue was purified with HPLC (Cosmosil 5SL-II ∅4.6 mm × 150 mm, eluent: toluene/$CHCl_3$/MeOH = 5/1/1, flow rate: 0.5 ml/min) to give **11a** (91% in Ref. [26]); vis (MeOH) λ_{max} = 652 (rel., 0.80), 608 (0.17), 426 (1.0), 326 nm (0.39); MS (FAB) found: m/z 805. Calcd for $C_{40}H_{47}N_5O_9{}^{64}Zn$: M^+, 805.

2.3.4. O-acetyl-galactosylated chlorophyll with ethylene spacer ***9***

Similar to the synthesis of **10a**, amidation of pyropheophorbide-*d* (40 mg) with **6** (153 mg) by EDC·HCl (56 mg) and HOBt (47 mg) in DMF (9 ml) and Et_3N (4 drops) for 20 h gave **7** (35.8 mg, 53%) after FCC purification.

tert-Butylamine borane complex (*t*BuNH_2BH_3, 10 mg) was added to a solution of **7** (30 mg) in CH_2Cl_2 (10 ml) at room temperature under N_2. After stirring for 1 h, the reaction mixture was washed with aqueous 2% HCl and aqueous 4% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated to dryness. The residue was purified with FCC (2–3% MeOH–$CHCl_3$), HPLC (Cosmosil 5C18-AR-II ∅4.6 mm × 150 mm, eluent: MeOH, flow rate: 1.0 ml/min) and recrystallization from MeOH/$CHCl_3$/CCl_4–hexane to give **9** (26 mg, 88%); vis (CH_2Cl_2) λ_{max} = 662 (rel., 0.47), 606 (0.08), 536 (0.09), 505 (0.10), 410 (1.0), 318 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.49, 9.45, 8.57 (each 1H, s, 5-, 10-, 20-H), 5.89, 5.82 (each 1H, d, J = 13 Hz, 3-CH_2), 5.28, 5.07 (each 1H, d, J = 19 Hz, 13^1-CH_2), 5.09 (1H, m, 4′-H), 4.72 (1H, dd, J = 8, 10 Hz, 2′-H), 4.62 (1H, dd, J = 4, 10 Hz, 3′-H), 4.49 (1H, q, J = 7 Hz, 18-H), 4.36 (1H, d, J = 7 Hz, 17-H), 3.72–3.62 (5H, m, 1′-OCH, 5′-, 8-CH_2), 3.64, 3.40, 3.24 (each 3H, s, 2-, 7-, 12-CH_3), 3.38 (1H, d, J = 8 Hz, 1′-H), 3.29–3.24 (2H, m, 1′-OCH, 5′-H), 3.00–2.50 (6H, m, 17-CH_2CH_2, 2′-H), 1.91, 1.89, 1.86, 1.52 (each 3H, s, $OCOCH_3$), 1.80 (3H, d, J = 7 Hz, 18-CH_3), 1.69 (3H, t, J = 7 Hz, 8^1-CH_3), 0.21, −1.86 (each 1H, s, NH); MS (ESI) found: m/z 912. Calcd for $C_{48}H_{58}N_5O_{13}$: $[M+H]^+$, 912.

2.3.5. Galactosylated chlorophyll with hexamethylene spacer ***10b***

Similar to the synthesis of **10a**, amidation of **12** (47 mg) with **5b** (85 mg) by EDC·HCl (63 mg) and HOBt (52 mg) in DMF (9 ml) for 19 h gave **10b** (32 mg, 46%) after FCC (10–12% MeOH–$CHCl_3$); vis (MeOH) λ_{max} = 660 (rel., 0.48), 604 (0.11), 536 (0.12), 504 (0.12), 407 (1.0), 319 nm (0.30); ^{1}H NMR (50% CD_3OD–$CDCl_3$) δ = 9.18, 9.16, 8.31 (each 1H, s, 5-, 10-, 20-H), 5.54 (2H, s, 3-CH_2), 5.01, 4.82 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.26 (1H, q, J = 8.5 Hz, 18-H), 4.01 (1H, d, J = 8 Hz, 17-H), 3.84 (1H, d, J = 7 Hz, 1′-H), 3.53–3.13 (12H, m, 1′-OCH_2, 2′-, 3′-, 4′-, 5′-H, 5′-, 8-CH_2, CH_2N), 3.41, 3.13, 2.97 (each 3H, s, 2-, 7-, 12-CH_3), 2.40–2.35, 2.15–2.10 (each 2H, m, 17-CH_2CH_2), 1.53 (3H, d, J = 8.5 Hz, 18-CH_3), 1.41 (3H, t, J = 8 Hz, 8^1-CH_3), 1.24–1.21 (2H, m, 1-$OCCH_2$), 1.00–0.87 (6H, m, $OC_2CH_2CH_2CH_2$); MS (ESI) found: m/z 799. Calcd for $C_{44}H_{57}N_5O_9$: M^+, 799.

2.3.6. Galactosylated zinc chlorophyll with hexamethylene spacer ***11b***

Similar to the synthesis of **11a**, zinc-metallation of **10b** (20 mg) in $CHCl_3$ (15 ml) and MeOH (5 ml) for 1 h gave **11b** (>90%) after HPLC (flow rate: 1.5 ml/min); vis (MeOH) λ_{max} = 652 (rel., 0.82), 607 (0.17), 426 (1.0), 325 nm (0.36); MS (APCI) found: m/z 862. Calcd for $C_{44}H_{56}N_5O_9{}^{64}Zn$: $[M+H]^+$, 862.

2.3.7. Galactosylated chlorophyll with decamethylene spacer ***10c***

Similar to the synthesis of **10a**, amidation of **12** (30 mg) with **5c** (70 mg) by EDC·HCl (41 mg) and HOBt (35 mg) in DMF (13 ml) for 19 h gave **10c** (28 mg, 56%) after FCC (10% MeOH–$CHCl_3$); vis (MeOH) λ_{max} = 660 (rel., 0.51), 604 (0.10), 536 (0.11), 504 (0.10), 407 (1.0), 319 nm (0.27); ^{1}H NMR (30% CD_3OD–$CDCl_3$) δ = 9.21, 9.20, 8.36 (each 1H, s, 5-, 10-, 20-H), 5.60 (2H, s, 3-CH_2), 5.06, 4.88 (each 1H, d, J = 20 Hz, 13^1-CH_2), 4.33 (1H, q, J = 8 Hz, 18-H), 4.07 (1H, d, J = 8 Hz, 17-H), 3.95 (1H, d, J = 7 Hz, 1′-H), 3.75–3.17 (12H, m, 1′-OCH_2, 2′-, 3′-, 4′-, 5′-H, 5′-, 8-CH_2, CH_2N), 3.38, 3.20, 3.03 (each 3H, s, 2-, 7-, 12-CH_3), 2.84–2.74, 2.17–2.12 (each 2H, m, 17-CH_2CH_2), 1.61 (3H, d, J = 8 Hz, 18-CH_3), 1.48 (3H, t, J = 8 Hz, 8^1-CH_3), 1.36–0.90 (16H, m, $OC(CH_2)_8$); MS (ESI) found: m/z 856. Calcd for $C_{48}H_{66}N_5O_9$: $[M+H]^+$, 856.

2.3.8. Galactosylated zinc chlorophyll with decamethylene spacer ***11c***

Similar to the synthesis of **11a**, zinc-metallation of **10c** (5 mg) in $CHCl_3$ (15 ml) and MeOH (5 ml) for 1 h gave **11c** (>90%) after HPLC (flow rate: 1.5 ml/min); vis (MeOH) λ_{max} = 652 (rel., 0.84), 607 (0.17), 426 (1.0), 325 nm (0.35); MS (ESI) found: m/z 916. Calcd for $C_{48}H_{62}N_5O_9{}^{64}Zn$: $[M-H]^-$, 916.

2.3.9. Galactosylated chlorophyll with dodecamethylene spacer ***10d***

Similar to the synthesis of **10a**, amidation of **12** (40 mg) with **5d** (96 mg) by EDC·HCl (54 mg) and HOBt (47 mg) in DMF (13 ml) for 19 h gave **10d** (37 mg, 56%) after FCC (12–14% MeOH–$CHCl_3$); vis (MeOH) λ_{max} = 660 (rel., 0.48), 604 (0.11), 536 (0.12), 504 (0.12), 407 (1.0), 319 nm (0.31); ^{1}H NMR (30% CD_3OD–$CDCl_3$) δ = 9.27, 9.25, 8.41 (each 1H, s, 5-, 10-, 20-H), 5.67 (2H, s, 3-CH_2), 5.10, 4.93 (each 1H, d, J = 20 Hz, 13^1-CH_2), 4.37 (1H, q, J = 8 Hz, 18-H), 4.03 (1H, d, J = 7 Hz, 17-H), 3.53–3.13 (13H, m, 1′-, 2′-, 3′-, 4′-, 5′-H, 1′-OCH_2, 5′-, 8-CH_2, CH_2N), 3.44, 3.28, 3.10 (each 3H, s, 2-, 7-, 12-CH_3), 2.90–2.20 (4H, m, 17-CH_2CH_2), 1.66 (3H, d, J = 8 Hz, 18-CH_3), 1.54 (3H, t, J = 8 Hz, 8^1-CH_3), 1.32–0.75 (20H, m, $OC(CH_2)_{10}$); MS (ESI) found: m/z 883. Calcd for $C_{50}H_{69}N_5O_9$: M^+, 883.

2.3.10. Galactosylated zinc chlorophyll with dodecamethylene spacer ***11d***

Similar to the synthesis of **11a**, zinc-metallation of **10d** (15 mg) in $CHCl_3$ (10 ml) and MeOH (5 ml) for 1 h gave **11d** (>90%) after HPLC (flow rate: 1.0 ml/min); vis (MeOH) λ_{max} = 652 (rel., 0.78), 607 (0.17), 426 (1.0), 323 nm (0.42); MS (ESI) found: m/z 944. Calcd for $C_{50}H_{66}N_5O_9{}^{64}Zn$: $[M-H]^-$, 944.

2.3.11. 3-Devinyl-3-hydroxymethyl-pyropheophorbide-a N-(11-carboxy-undecamethylene)amide (***13***)

Similar to the synthesis of **10a**, amidation of **12** (30 mg) with methyl 12-aminododecanoate hydrochloride (14 mg) by EDC·HCl (41 mg) and HOBt (35 mg) in DMF (8 ml) and Et_3N (4 drops) for 18 h gave the corresponding amide (26.5 mg, 63%) after FCC (4–5% MeOH–CH_2Cl_2) and recrystallization from CH_2Cl_2–hexane; vis (CH_2Cl_2) λ_{max} = 662 (rel., 0.47), 606 (0.08), 536 (0.12), 505 (0.10), 410 (1.0), 317 nm (0.20); ^{1}H NMR ($CDCl_3$) δ = 9.47, 9.44, 8.50 (each

1H, s, 5-, 10-, 20-H), 5.90 (2H, s, 3-CH_2), 5.20, 5.08 (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.50 (1H, q, J = 7.5 Hz, 18-H), 4.03 (1H, d, J = 8 Hz, 17-H), 3.69 (2H, q, J = 8 Hz, 8-CH_2), 3.63, 3.62, 3.41, 3.26 (each 3H, s, 2-, 7-, 12-CH_3, $COOCH_3$), 2.99–2.86 (2H, m, NCH_2), 2.67–2.39 (4H, m, 17-CH_2CH_2), 2.23 (2H, t, J = 8 Hz, $COCH_2$), 1.78 (3H, d, J = 7.5 Hz, 18-CH_3), 1.69 (3H, t, J = 8 Hz, 8^1-CH_3), 1.25–0.99 (18H, m, $COC(CH_2)_9$), 0.26, −1.81 (each 1H, s, NH); MS (ESI) found: m/z 750. Calcd for $C_{45}H_{60}N_5O_5$: $[M+H]^+$, 750.

Similar to the synthesis of **12**, hydrolysis of the above methyl ester (26 mg) in a small amount of acetone and concentrated HCl (20 ml) for 2 h gave **13** (25 mg, 98%) after recrystallization from $CHCl_3$/MeOH–hexane; vis (MeOH) λ_{max} = 660 (rel., 0.49), 604 (0.10), 536 (0.11), 505 (0.11), 407 (1.0), 319 nm (0.25); ^{1}H NMR (20% CD_3OD–$CDCl_3$) δ = 9.40, 9.39, 8.49 (each 1H, s, 5-, 10-, 20-H), 5.81 (2H, s, 3-CH_2), 5.17, 5.03 (each 1H, d, J = 20 Hz, 13^1-CH_2), 4.46 (1H, q, J = 6.5 Hz, 18-H), 4.24 (1H, d, J = 8 Hz, 17-H), 3.64 (2H, q, J = 8 Hz, 8-CH_2), 3.56, 3.36, 3.21 (each 3H, s, 2-, 7-, 12-CH_3), 2.93–2.85 (2H, m, NCH_2), 2.67–2.39 (4H, m, 17-CH_2CH_2), 2.23 (2H, t, J = 8 Hz, $COCH_2$), 1.74 (3H, d, J = 6.5 Hz, 18-CH_3), 1.65 (3H, t, J = 8 Hz, 8^1-CH_3), 1.48 (2H, t, J = 7 Hz, NCH_2), 1.25–0.99 (18H, m, $COC(CH_2)_9$); MS (ESI) found: m/z 734. Calcd for $C_{44}H_{56}N_5O_5$: $[M-H]^-$, 734.

2.3.12. Galactosylated chlorophyll with undecamethylene-CONH-ethylene spacer ***14a***

Similar to the synthesis of **10a**, amidation of **13** (25 mg) with **5a** (70 mg) by EDC·HCl (35 mg) and HOBt (29 mg) in DMF (9 ml) for 19 h gave **14a** (19.5 mg, 59%) after FCC (10–15% MeOH–$CHCl_3$); vis (MeOH) λ_{max} = 660 (rel., 0.50), 605 (0.09), 536 (0.10), 505 (0.10), 407 (1.0), 319 nm (0.27); ^{1}H NMR (40% CD_3OD–$CDCl_3$) δ = 9.20, 9.16, 8.36 (each 1H, s, 5-, 10-, 20-H), 5.60 (2H, s, 3-CH_2), 5.05, 4.87, (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.33 (1H, q, J = 8 Hz, 18-H), 4.20–3.18 (12H, 17-H, 1′-, 2′-, 3′-, 4′-, 5′-H, 1′-OCH_2, 5′-, 8-CH_2), 2.90–2.84 (2H, m, CH_2N), 3.36, 3.20, 3.04 (each 3H, s, 2-, 7-, 12-CH_3), 2.91–2.71 (2H, m, NCH_2), 2.50–2.40, 2.20–2.10 (each 2H, m, 17-CH_2CH_2), 2.06 (2H, t, J = 7 Hz, $NCOCH_2$), 1.62 (3H, d, J = 8 Hz, 18-CH_3), 1.49 (3H, t, J = 8 Hz, 8^1-CH_3), 1.10–0.94 (18H, m, $COC(CH_2)_9$); MS (ESI) found: m/z 941. Calcd for $C_{52}H_{73}N_6O_{10}$: $[M+H]^+$, 941.

2.3.13. Galactosylated zinc chlorophyll with undecamethylene-CONH-ethylene spacer ***15a***

Similar to the synthesis of **11a**, zinc-metallation of **14a** (15 mg) in $CHCl_3$ (5 ml) and MeOH (15 ml) for 50 min gave **15a** (>90%) after HPLC (flow rate: 0.5 ml/min); vis (MeOH) λ_{max} = 652 (rel., 0.82), 607 (0.17), 426 (1.0), 325 nm (0.38); MS (ESI) found: m/z 1001. Calcd for $C_{52}H_{69}N_6O_{10}{}^{64}Zn$: $[M-H]^-$, 1001.

2.3.14. Galactosylated chlorophyll with undecamethylene-CONH-hexamethylene spacer ***14b***

Similar to the synthesis of **10a**, amidation of **13** (30 mg) with **5b** (45 mg) by EDC·HCl (43 mg) and HOBt (29 mg) in DMF (12 ml) for 19 h gave **14b** (21 mg, 52%) after FCC (12–20% MeOH–$CHCl_3$); vis (MeOH) λ_{max} = 660 (rel., 0.50), 604 (0.10), 536 (0.11), 504 (0.11), 407 (1.0), 319 nm (0.28); ^{1}H NMR (30% CD_3OD–$CDCl_3$) δ = 9.28, 9.22, 8.42 (each 1H, s, 5-, 10-, 20-H), 5.68, 5.66 (each 1H, d, J = 13 Hz, 3-CH_2), 5.09, 4.94, (each 1H, d, J = 19 Hz, 13^1-CH_2), 3.89 (1H, q, J = 7 Hz, 18-H), 4.14 (1H, br-d, J = 5 Hz, 17-H), 4.04 (1H, d, J = 8 Hz, 1′-H), 3.77 (1H, d, J = 2 Hz, 4′-H), 3.73 (1H, dt, J = 10, 7 Hz, 1′-OCH), 3.70–3.27 (8H, m, 1′-OCH, 2′-, 3′-, 5′-H, 5′-, 8-CH_2), 3.01–2.98 (2H, m, CH_2N) 3.41, 3.27, 3.12 (each 3H, s, 2-, 7-, 12-CH_3), 2.93–2.82 (2H, m, CH_2N), 2.53–2.48, 2.21–2.15 (each 2H, m, 17-CH_2CH_2), 1.92 (2H, t, J = 7 Hz, $NCOCH_2$), 1.68 (3H, d, J = 7 Hz, 18-CH_3), 1.56 (3H, t, J = 8 Hz, 8^1-CH_3), 1.40–1.00 (26H, m, $OC(CH_2)_4$, $COC(CH_2)_9$); MS (ESI) found: m/z 1019. Calcd for $C_{56}H_{80}N_6O_{10}Na$: $[M+Na]^+$, 1019.

2.3.15. Galactosylated zinc chlorophyll with undecamethylene-CONH-hexamethylene spacer ***15b***

Similar to the synthesis of **11a**, zinc-metallation of **14b** (5 mg) in $CHCl_3$ (3 ml) and MeOH (6 ml) for 50 min gave **15b** (>90%) after HPLC (flow rate: 1.0 ml/min); vis (MeOH) λ_{max} = 652 (rel., 0.84), 606 (0.18), 426 (1.0), 325 nm (0.41); MS (ESI) found: m/z 1057. Calcd for $C_{56}H_{77}N_6O_{10}{}^{64}Zn$: $[M-H]^-$, 1057.

2.3.16. Galactosylated chlorophyll with undecamethylene-CONH-decamethylene spacer ***14c***

Similar to the synthesis of **10a**, amidation of **13** (25 mg) with **5c** (45 mg) by EDC·HCl (35 mg) and HOBt (29 mg) in DMF (10 ml) for 19 h gave **14c** (21 mg, 59%) after FCC (10–17% MeOH–$CHCl_3$); vis (MeOH) λ_{max} = 660 (rel., 0.50), 603 (0.10), 536 (0.11), 505 (0.11), 407 (1.0), 319 nm (0.29); ^{1}H NMR (30% CD_3OD–$CDCl_3$) δ = 9.22, 9.21, 8.37 (each 1H, s, 5-, 10-, 20-H), 5.61 (2H, s, 3-CH_2), 5.06, 4.89, (each 1H, d, J = 19 Hz, 13^1-CH_2), 4.34 (1H, q, J = 8 Hz, 18-H), 4.09 (1H, d, J = 7 Hz, 17-H), 4.01 (1H, d, J = 7 Hz, 1′-H), 3.70 (1H, d, J = 2 Hz, 4′-H) 3.68 (1H, dt, J = 10, 7 Hz, 1′-OCH), 3.62–3.27 (8H, m, 1′-OCH, 2′-, 3′-, 5′-H, 5′-, 8-CH_2), 2.96–2.92 (2H, m, CH_2N) 3.39, 3.21, 3.05 (each 3H, s, 2-, 7-, 12-CH_3), 2.86–2.76 (2H, m, CH_2N), 2.48–2.45, 2.19–2.12 (each 2H, m, 17-CH_2CH_2), 1.89 (2H, t, J = 7 Hz, $CNCOCH_2$), 1.62 (3H, d, J = 8 Hz, 18-CH_3), 1.49 (3H, t, J = 8 Hz, 8^1-CH_3), 1.34–0.94 (34H, m, $OC(CH_2)_8$, $COC(CH_2)_9$); MS (ESI) found: m/z 1052. Calcd for $C_{60}H_{88}N_6O_{10}$: M^+, 1052.

2.3.17. Galactosylated zinc chlorophyll with undecamethylene-CONH-decamethylene spacer ***15c***

Similar to the synthesis of **11a**, zinc-metallation of **14c** (5 mg) in $CHCl_3$ (15 ml) and MeOH (5 ml) for 1 h gave **15c** (>90%) after HPLC (flow rate: 1.0 ml/min); vis (MeOH) λ_{max} = 652 (rel., 0.87), 607 (0.17), 426 (1.0), 325 nm (0.34); MS (APCI) found: m/z 1115. Calcd for $C_{60}H_{87}N_6O_{10}{}^{64}Zn$: $[M+H]^+$, 1115.

2.3.18. Galactosaminated zinc chlorophyll with undecamethylene-CO spacer ***16***

Similar to the synthesis of **10a**, amidation of **13** (27 mg) with galactosamine hydrochloride (25 mg) by EDC·HCl (28 mg) and HOBt (23 mg) in DMF (10 ml) and Et_3N (3 drops) for 18 h gave the corresponding amide after FCC (10–15% MeOH–$CHCl_3$).

Similar to the synthesis of **11a**, zinc-metallation of the above free base (5 mg) in $CHCl_3$ (10 ml) and MeOH (5 ml) for 50 min gave **16** (>90%) after reverse-phase HPLC (Inertsil ø4.6 mm × 150 mm, eluent: CH_3CN/MeOH/$CHCl_3$ = 5/1/1, flow rate: 1.0 ml/min).

2.4. Preparation of solutions of synthetic zinc chlorophylls

Synthetic zinc chlorophylls **1**, **11a**–**d** and **15a**–**c** were dissolved in methanol at the concentration of ca. 1 mM. The methanol solution (50 μl) was poured into distilled water to give its aqueous solution (5 ml): all the final concentrations of chlorophylls were about 10 μM. After being shaken vigorously, the aqueous solution containing 1% (v/v) methanol was allowed to stand for over 1 h at room temperature and its electronic absorption spectra were measured. In the case of **1**, octyl β-D-galactoside (ca. 0.1 M) was added to the initial methanol solution. The concentrated methanol solution of zinc chlorophylls at the same concentration (ca. 1 mM) was diluted with methanol to make their monomeric solution (5 ml, about 10 μM).

2.5. Spectral analysis by deconvolution

Electronic absorption spectra in the region over 540 nm were fitted by five Gaussian-curved bands. First, the spectra were transformed by an energy level unit: from wavelength (nm) to

wavenumber (cm^{-1}). After correction of a base line, the curve fitting was performed by a calculation software, Igor Pro 3.16 (Wavemetrics Inc.). The deconvolution spectra are shown in Fig. S1 of Supplementary data.

3. Results and discussion

3.1. Synthesis of zinc 3-hydroxymethyl-13^1-oxo-chlorins possessing a galactosyl moiety in the 17-substituent

To prepare desired galactosylated zinc bacteriochlorophyll-*d* analogs, the effective linkage of a chlorin moiety with a galactosyl moiety is necessary. Ester linkage at the 17-propionate residue is one of the useful preparations of various chlorophyllous conjugates: 17^2-COOH + HOR → 17^2-COOR. In the present case, galactose has five reactive hydroxy groups in a molecule and the 1-hydroxy group can be selectively esterified after protection of the other hydroxy groups. Usually, the protection is performed by esterification including acetylation and benzoylation, and their selective deprotection is difficult in the resulting 17^2-COOR. Therefore, amidation of the 17-propionic acid residue was used for preparation of the desired conjugates. First, β-galactose ether-linked with an amino-terminated oligomethylene group at the 1-position was prepared. Usual conditions for deprotection giving hydroxy groups in galactosides are harmful for chlorophyll derivatives having several reactive functional groups including the 13-keto carbonyl group. Therefore, the unprotected amino-galactose was selectively condensed with pyropheophorbides possessing 17^2-COOH to give desired 17^2-CONHR.

3.1.1. Synthesis of ω-aminoalkyl β-galactosides

Desired amino-galactoses **5** were prepared as shown in Scheme 1. The synthetic route has already been reported [36] and is briefly reported here. Commercially available β-D-galactose pentaacetate [Gal(OAc)$_5$] in dichloromethane was treated with 2-bromo-ethanol in the presence of boron trifluoride etherate (see step (i)). After stirring overnight under nitrogen, the reaction mixture was purified with FCC over silica gel to give 1-(2-bromoethyl) ether as the isolable product. The major separated product was β-isomer **2a** (68% yield), while its α-isomer could be isolated as a minor product. Using ω-bromo-1-alkanols, Br-(CH$_2$)$_n$-OH (n = 6, 10 and 12), the corresponding stereochemically pure ω-bromo-alkyl ethers **2b**–**d** were given in moderate yields. The resulting bromides **2a**–**d** were reacted with sodium azide in DMF to afford their azides **3a**–**d** almost quantitatively (step (ii)). After deprotection of tetraacetates **3a**–**d** by sodium methoxide in methanol (step (iii), in almost quantitative yields), the resulting azides **4a**–**d** were hydrogenated on platinum oxide to give ω-aminoalkyl β-galactosides **5a**–**d** almost quantitatively (step (iv)). During the above modifications after isolation of **2**, no change of the stereochemistry was observed in ^{1}H NMR analyses.

O-tetraacetate of **5a** was prepared from hydrogenation of azide **3a** on Lindlar catalyst and its *p*-toluenesulfonate salt **6** was obtained in 95% yield. The acetate moieties were readily reacted with any free amino group in a concentrated solution and the hydrogenation had to be done under acidic conditions. The resulting salt **6** was stored and neutralized just before the following amidation (vide infra).

3.1.2. Synthesis of β-galactosylated chlorophyll derivatives

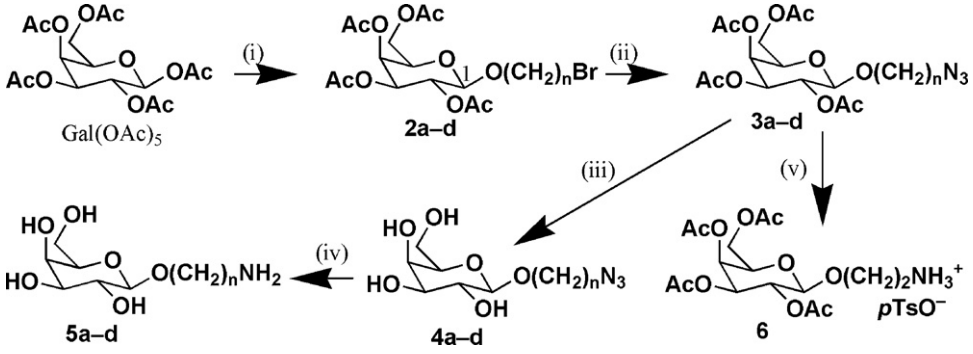
The 17^2-carboxy group of pyropheophorbide-*d* was reacted with the amino group of neutralized **6** by action of carbodiimide to give the corresponding amide **7** in 53% yield (see the top right step in Scheme 2). Basic deprotective cleavage of tetraacetate moieties in **7** was applied (step (iv)), but the desired product **8** could not be isolated from the complex reaction mixture. This unavailability was ascribed to the instability of the pyropheophorbide moiety under the basic conditions (vide supra). Selective reduction of the 3-formyl group in **7** gave 3-hydroxymethyl-chlorin **9** in 88% yield (step (iii)). After treatment of **9** under the same basic conditions, fully unprotected form **10a** could not be obtained at all.

Pyropheophorbide-*d* possessing the 3-formyl group in DMF was amidated with *O*-unprotected galactoside **5a** by water-soluble carbodiimide (EDC). In the presence of HOBt, the EDC-coupling of the carboxy group in pyropheophorbide-*d* with the amino group in **5a** smoothly proceeded to give **8**, but no undesired esterification of the former acid component with the hydroxy groups in **5a** was observed. Pure amide **8** could, unfortunately, not be separated from the reaction mixture. After the reduction (step (iii)) of the crude product containing **8** followed by zinc-metallation (step (v)), **11a** was successfully isolated by purification with HPLC. The total yield from pyropheophorbide-*d* to **11a** was less than 10%.

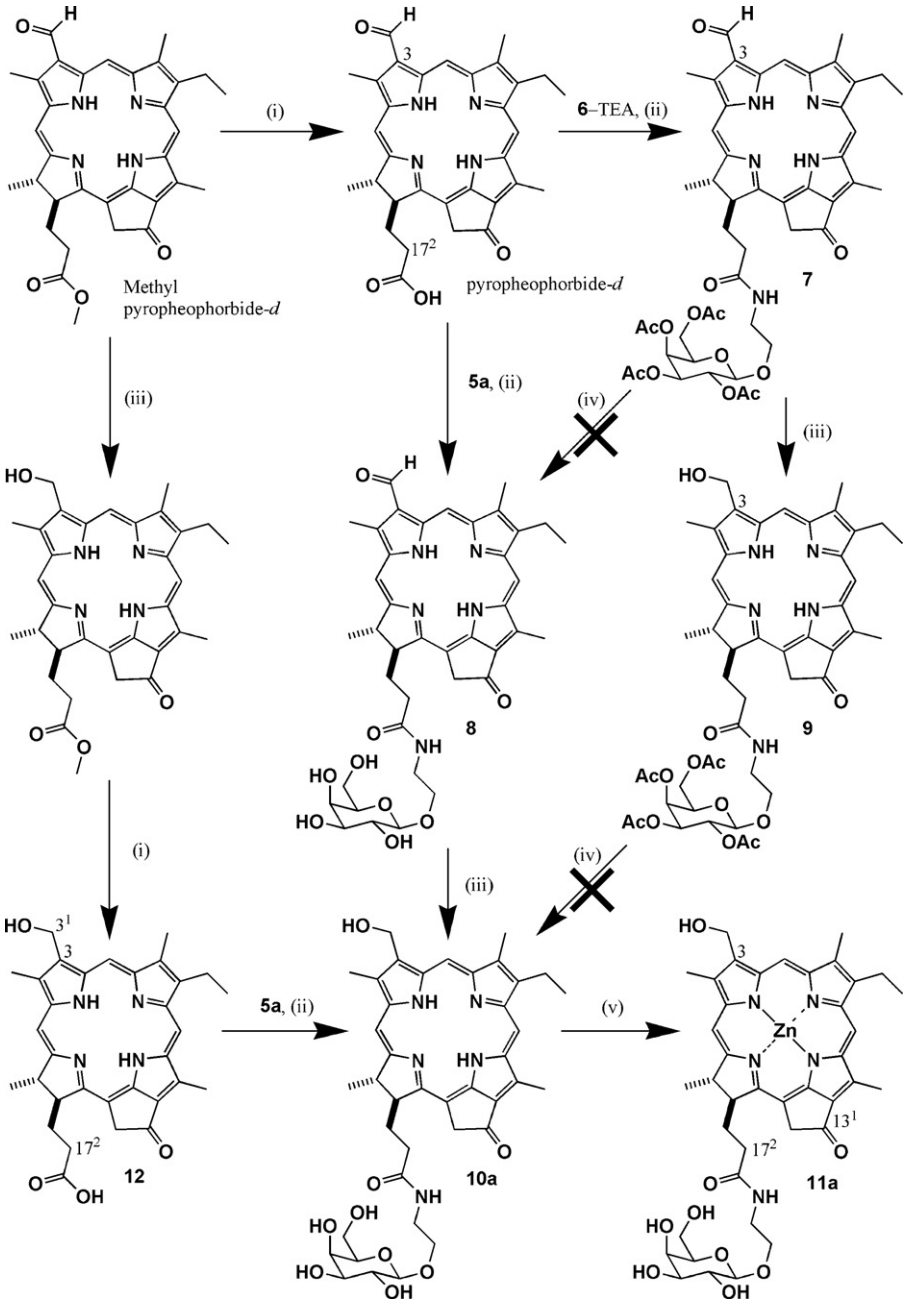
Coupling of 3-hydroxymethyl-pyropheophorbide **12** with **5a** was applied by action of EDC–HOBt. The desired amidation was observed successfully to give pure **10a** in 56% yield (bottom left step in Scheme 2), but the 17^2-carboxy group in **12** was not esterified with any hydroxy groups in **5a** or 3^1-OH in **12**. After zinc-metallation of free base **10a** (step (v)), β-D-galactosylated zinc 3-hydroxymethyl-13^1-oxo-chlorin **11a** was obtained in 91% yield. The above results indicated that EDC–HOBt coupling was useful for amidation of any pyropheophorbides with amines even in the presence of hydroxy groups in the reactants, and that an amino group was more reactive under the coupling conditions than a hydroxy group and exclusively reacted with the activated carboxylic acid.

3.1.3. Synthesis of galactosylated zinc bacteriochlorophyll-d analogs

As mentioned above, acid component **12** was condensed with unprotected amino-galactose **5b**–**d** by EDC–HOBt to give



Scheme 1. Synthesis of amino-galactosides **5** and **6** (**a**: n = 2, **b**: n = 6, **c**: n = 10, **d**: n = 12): (i) HO(CH$_2$)$_n$Br–BF$_3$OEt$_2$/CH$_2$Cl$_2$; (ii) NaN$_3$/DMF; (iii) MeONa/MeOH; (iv) H$_2$–PtO$_2$/MeOH; (v) H$_2$–Lindlar catalyst–*p*TsOH/EtOH.



Scheme 2. Synthesis of galactosylated zinc chlorophyll **11a**: (i) conc. HCl; (ii) EDC·HCl–HOBt/DMF; (iii) tBuNH$_2$BH$_3$/CH$_2$Cl$_2$; (iv) MeONa/MeOH; (v) Zn(OAc)$_2$2H$_2$O/MeOH–CH$_2$Cl$_2$.

the corresponding amides **10b–d** in comparable yields (see Scheme 3). Zinc-metallation of **10b–d** afforded **11b–d** (>90% yield). The increase in length in their oligomethylene spacer (n = 2 → 6 → 10 → 12) did not affect the coupling or metallation.

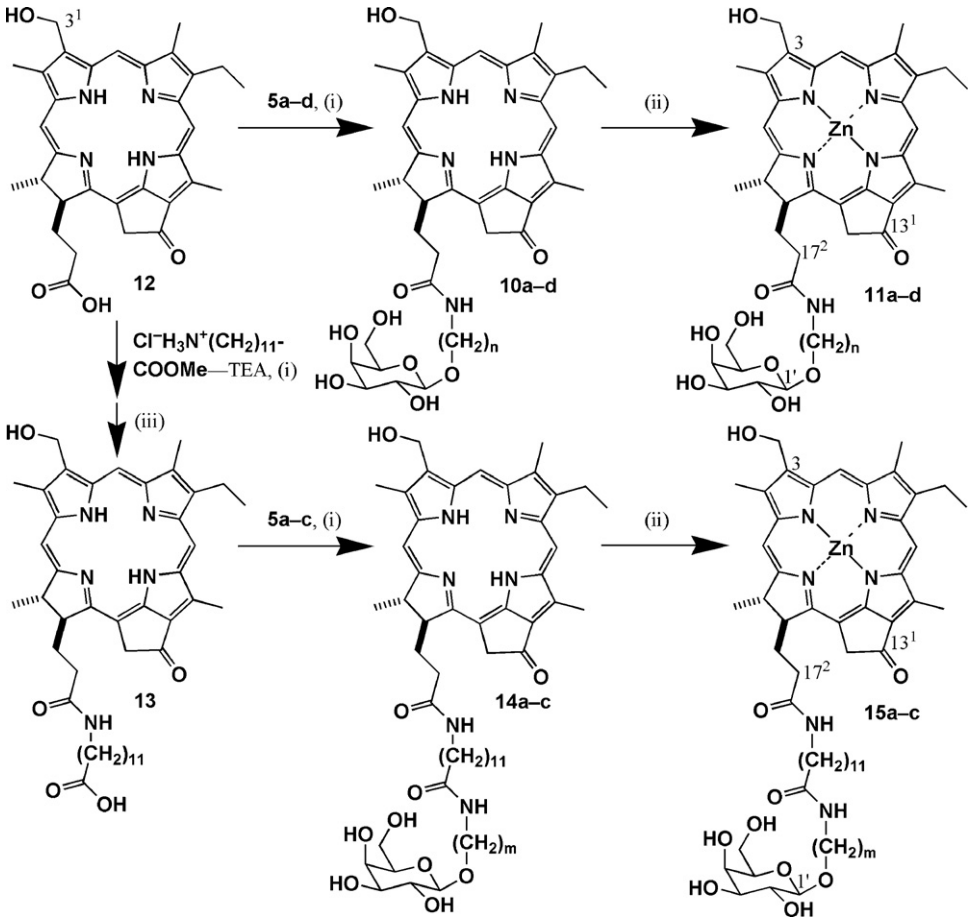
To enlarge the length of spacer between chlorin and galactosyl moieties, 12-amino-dodecanoic acid was inserted to **11a–c**. First, **12** was amidated with methyl 12-amino-dodecanoate and the methyl ester in the resulting amide was hydrolyzed under acidic conditions (step (iii)) to give **13** in 62% yield for the two steps. Further amidation of **13** with **5a–c** afforded **14a–c** (>50%) and the successive zinc-metallation gave **15a–c** (>90%). The atom numbers in the linked spacer between 17^2-CONH of zinc bacteriochlorophyll-*d* analog and 1′-O of β-galactose were 2, 6, 10, 12, 15, 19 and 23 for **11a** (n = 2), **11b** (n = 6), **11c** (n = 10), **11d** (n = 12), **15a** (m = 2), **15b** (m = 6) and **15c** (m = 10), respectively.

Similar to the synthesis of **15**, acid **13** was condensed with galactosamine and zinc-metallated to give the other galactosylated zinc bacteriochlorophyll-*d* analog **16** (see the left drawing of Fig. 2). The carbon atom number (12) of the linkage between 17^2-CONH of chlorin moiety and 2′-NH of galactosyl moiety in **16** is the same as that between 17^2-CONH of chlorin moiety and 1′-O of galactosyl moiety in **11d**.

3.2. Electronic absorption spectra of synthetic zinc chlorophylls

3.2.1. In methanol

All the synthetic zinc chlorophyll derivatives **1**, **11**, **15** and **16** examined here were dissolved in methanol and the solutions gave the same electronic absorption spectra (see broken line of Fig. 3). At 652 nm, a sharp band was observed to be assigned to Q_y band



Scheme 3. Synthesis of galactosylated zinc chlorophylls **11** and **15** possessing oligomethylene spacers (**a**: n or $m = 2$, **b**: n or $m = 6$, **c**: n or $m = 10$, **d**: $n = 12$): (i) EDC·HCl–HOBt/DMF; (ii) $Zn(OAc)_2 2H_2O$/MeOH–CH_2Cl_2; (iii) conc. HCl/acetone.

and its full-width at half-maximum (FWHM) was 420 cm^{-1}. The other intense band was seen at a shorter wavelength and the 426-nm maximum was assigned to the Soret band. The intensity of Q_y peak was slightly less than that of Soret peak and the ratio of the former over the latter was 0.82. These spectral features showed that the chlorophyllous pigments were monomeric in methanol, where their central zinc was axial-ligated to a methanol molecule to take a 5-coordinated state [23]. The lack of spectral change in a series of zinc chlorophylls indicated that 17^2-substituents did not affect intramolecularly the chlorin π-system [37] in a diluted methanol solution (10 μM) at room temperature.

3.2.2. *In an aqueous solution of octyl β-D-galactoside*

A methanol solution of methyl ester **1** was diluted with an excess of water to give precipitates quickly due to its high hydrophobicity [38]. The solids prepared were amorphous aggregates of **1** as

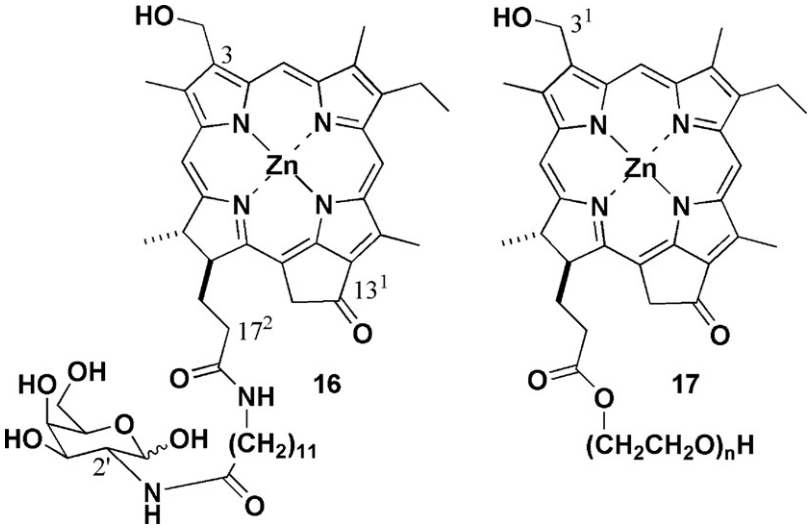


Fig. 2. Molecular structures of zinc bacteriochlorophyll-*d* analogs covalently linked with galactosamine **16** and oligoethylene glycol **17**.

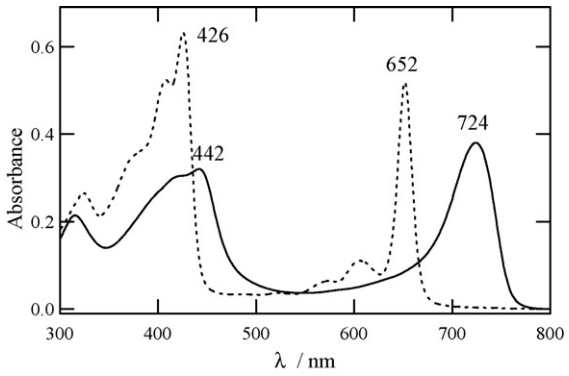


Fig. 3. Electronic absorption spectra of zinc bacteriochlorophyll-*d* analog **1** (ca. 10 μM) and octyl β-D-galactoside (ca. 1 mM) in MeOH (broken) and 1% (v/v) MeOH–H_2O (solid).

often seen in other chlorophyllous pigments. In the presence of octyl β-D-galactoside, the aqueous solution remained stable and green colored after standing for a few days. The electronic absorption spectrum shows red-shifted and broadened bands compared with the monomeric one in methanol (Fig. 3). The values in red-shift of Q_y and Soret bands were 1530 and 860 cm^{-1}, respectively, and the FWHM of Q_y band increased from 420 to 1080 cm^{-1} by dilution with water. Moreover, the intensity of Q_y maximum was larger than that of Soret and the ratio, Abs(Q_y)/Abs(Soret) enhanced about 50% from 0.82 to 1.19 with the dilution. The spectrum observed in the aqueous solution of **1** was similar to that of natural chlorosomes in an aqueous buffer solution [23]. Therefore, methyl ester **1** formed chlorosomal self-aggregates inside hydrophobic environments prepared by self-assembly of octyl galactoside and a large oligomer of **1** was present in an aqueous micelle of octyl galactoside. As reported previously [5,23,39], the supramolecular structure of oligomeric **1** was constructed by specific bonding of Zn···O(3^1)–H···O=C(13^1) and π–π stacking of chlorin chromophores and such a well-ordered J-aggregation gave the above spectral changes.

3.2.3. *In an aqueous methanol solution*

In contrast with methyl ester **1** (vide supra), more hydrophilic zinc chlorophyll derivatives **11**, **15** and **16** possessing a galactosyl moiety were soluble in an aqueous 1% (v/v) methanol solution and their 10 μM solution gave no precipitates after standing for a few days at room temperature. Their electronic absorption spectra are shown in Fig. 4.

Compounds **11a** possessing the shortest ethylene linker and **11b** possessing the second shortest hexamethylene linker gave similar spectra. The main Q_y band was situated at 677–678 nm and

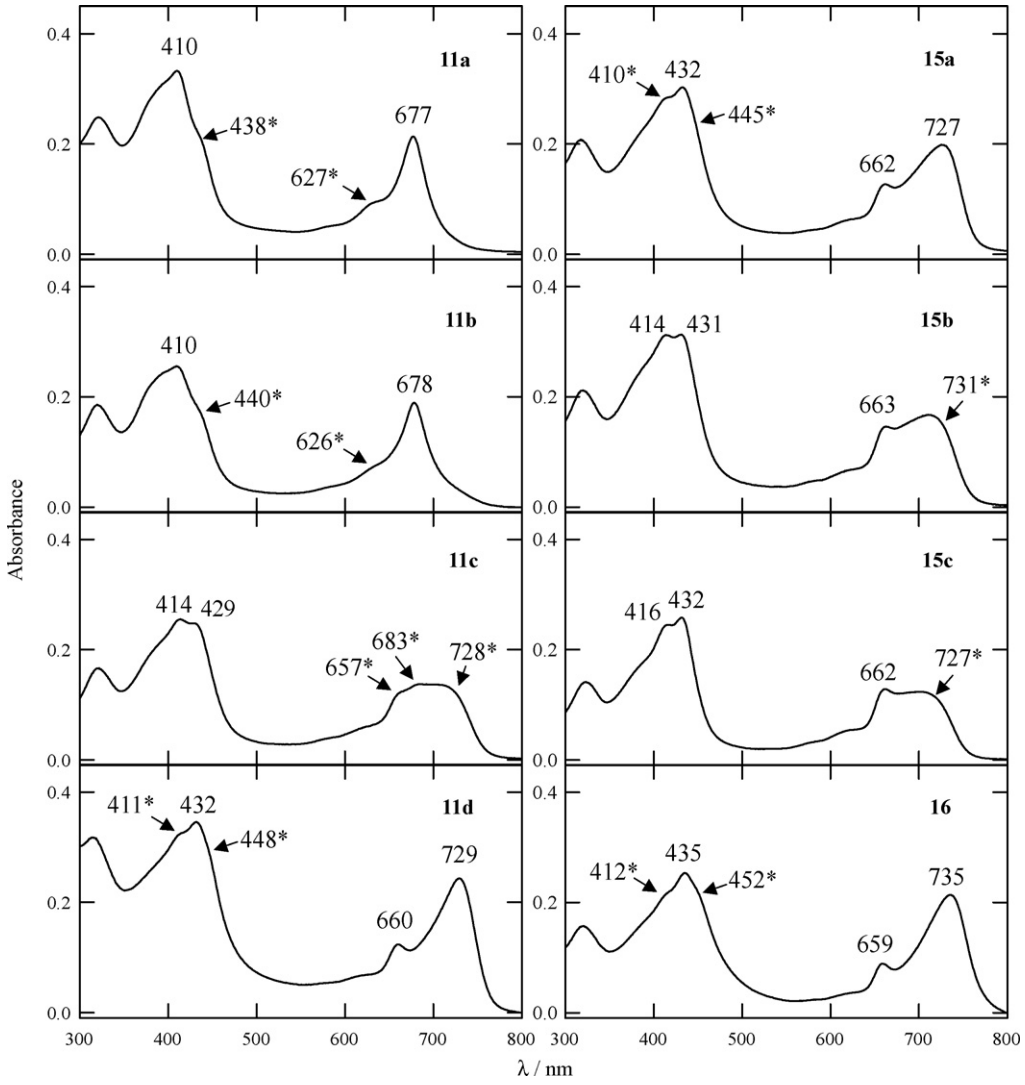


Fig. 4. Electronic absorption spectra of galactosylated zinc chlorophylls **11**, **15** and **16** (ca. 10 μM) in 1% (v/v) MeOH–H_2O (solid): for **11a**, **11b**, **11c** and **11d** from top to bottom of left and for **15a**, **15b**, **15c** and **16** from top to bottom of right. The values with asterisk were estimated from the second derivative (see Fig. S2).

Table 1
Deconvolution fitting peaks λ_{max} (nm) with FWHM (cm^{-1}) for Q_y bands of galactosyl–chlorophyll conjugates in 1% (v/v) methanol and water, and their relative intensity (%)[a].

Compound[b]	λ_{max} (FWHM) [relative peak intensity]		
	A1 <monomer>	A2 <dimer>	A3 <oligomer>
11a (2)	665 (375) [31]	681 (320) [55]	704 (389) [14]
11b (6)	666 (416) [26]	682 (341) [54]	709 (569) [20]
11c (10)	662 (403) [26]	685 (406) [29]	718 (564) [45]
11d (12)	659 (378) [17]	696 (483) [28]	730 (421) [55]
15a (15)	661 (414) [23]	698 (491) [32]	731 (418) [45]
15b (19)	660 (389) [28]	691 (472) [33]	723 (460) [39]
15c (23)	660 (356) [29]	687 (497) [37]	721 (473) [34]
16 (12)	659 (364) [18]	699 (579) [29]	737 (470) [53]

[a] Three peaks at >650 nm are shown.
[b] In parentheses are indicated the atom numbers in the linked spacer between 17^2-CONH of zinc bacteriochlorophyll-*d* analog and galactosyl moiety.

the maximum was shifted to a longer wavelength than that of monomer in methanol (652 nm) and to a shorter wavelength than that of the above large oligomer in an aqueous solution of octyl galactoside (724 nm). At the blue side of the main Q_y bands, a shoulder was observed clearly. The position was situated at around 626–627 nm which was a shorter wavelength than the monomeric peak at 652 nm. It is reported that zinc chlorophylls **17** possessing oligo(ethylene glycol) moieties (see the right drawing of Fig. 2) dimerized in head-to-tail and slipped cofacial manners by mutual coordination of the 3^1-OH of a molecule with the central Zn of the other to give 675-nm absorbing species with a 630-nm shoulder in 1% MeOH–H_2O [2]. As a result, both **11a** and **11b** formed primarily similar dimers as **17** in an aqueous methanol solution.

For **11c** possessing a decamethylene spacer, a broad Q_y band was observed at around 650–750 nm. The second derivative of the spectrum in the Q_y region showed the presence of at least three comparable components: 657, ca. 680 and ca. 730 nm (see Fig. S2, Supplementary data). The 657-nm absorbing species is assigned to the monomer [2] and the position was shifted hypsochromically from 652 nm in methanol due to the solvent change. The 680-nm species is assigned to the dimer described above. The 730-nm species is assigned to an oligomer based on chlorosomal J-aggregates as shown in **1**.

The spectrum of **11d** possessing a dodecamethylene spacer shows that the oligomeric and redmost Q_y species at 729 nm was major and the monomeric species was clearly observed as a 660-nm peak. Insertion of $CONHCH_2$ to the linker of **11d** as in **15a** relatively increased the absorbance at the region between oligomeric and monomeric peaks. The same tendency was observed in the insertion of a tetramethylene group from **15a** to **15b** and from **15b** to **15c**.

The electronic absorption spectrum of **16** possessing $(CH_2)_{11}CONH$ spacer is similar to that of **11d** possessing $(CH_2)_{12}O$ spacer. Both the spacers have 12 carbon atoms and their lengths are almost the same. Such C12 linkers were effective for chlorosomal self-aggregation of zinc chlorophyll derivatives covalently linked with galactosyl moiety in an aqueous methanol solution, and shorter and longer spacers suppressed formation of the J-aggregates absorbing >700 nm.

3.2.4. Spacer-dependent electronic absorption bands

To investigate the Q_y-absorbing species more deeply, deconvolution analysis of absorption spectra at >540 nm was performed (see Fig. S1). The analytical data are summarized in Table 1. For all of them, three peaks A1, A2 and A3 were obtained at >650 nm. The A1 peaks at the shortest wavelength were situated from 659 to 666 nm and their FWHMs were $387 \pm 23\ cm^{-1}$, indicating their assignation to monomeric species as mentioned above. The positions of 659–662 nm in the A1 of **11c**/**d**, **15a**–**c**, and **16** were in good agreement with the maximum (657 nm) of the monomeric peak proposed above. The A2 bands gave maxima at 681–699 nm and could be come from small aggregates including dimer. Compared with the above assignment, both **11a** and **11b** mainly (>50% intensity) gave a head-to-tail type dimeric peak at 681–682 nm. The A3 peaks at the longest wavelength moved from 704 to 737 nm and their FWHMs were also greatly changed: 1.5-fold enhancement from 390 to 570 cm^{-1}. Therefore, the A3 bands came from various oligomers and the major A3 peaks at $\geq$730 nm in **11d**, **15a**, and **16** would be assigned to chlorosomal J-aggregates (vide supra).

Comparison of the relative peak intensities in each deconvoluted band showed the values in A1 and A2 decreased with an increase of linker length in **11a**–**d** and increased in **11d** $\approx$ **16** < **15a** < **15b** < **15c**. The intensities in A3 had the reverse dependency on length: **11a** < **11b** < **11c** < **11d** $\approx$ **16** > **15a** > **15b** > **15c**. A similar order is observed in the peak positions of A3: **11a** < **11b** < **11c** < **11d** < **16** > **15a** > **15b** > **15c**. These results indicated that the chlorophyll–galactose conjugates **11d** and **16** with 12 carbon atoms as the linked core were the most useful for preparation of chlorosomal large J-aggregates in an aqueous methanol solution, and that shortening and prolonging the spacer from the 12-atom linker gradually increased the proportions of monomer and small aggregates and decreased that of large self-aggregates. Considering that the aggregation number would be proportional to the absorption maximum, these numbers increased in **11a** < **11b** < **11c** < **11d** and decreased in **15a** > **15b** > **15c** and the largest aggregate was formed in **16**.

The maximum formation of large aggregates in the 12-atom spacer can be explained as follows. Synthetic zinc bacteriochlorophyll-*d* analogs readily self-aggregate by specific bonds of Zn···O(3^1)–H···O=C(13^1) and π–π stacks of chlorins to form large oligomers (vide supra). The resulting well-ordered J-aggregates have a hydrophobic core part which would be covered with 17-substituents of the supramolecules. In an aqueous solution, such a relatively rigid core part must be surrounded with hydrophilic moieties to form the aqueous supramolecule. The relatively flexible peripheral part interacted with environmental water molecules, and the aggregates were stabilized and solubilized in an aqueous solution without further self-assembling to make precipitates. In the present systems, a galactosyl group in the terminal of the 17-substituent is hydrophilic and can interact with water molecules. Shorter oligomethylene linkers are less flexible and the terminal galactosyl groups have difficulty in taking good conformations to stabilize the aqueous supramolecule. Longer hydrophobic linkers interact with each other (intermolecularly) and with themselves (intramolecularly) largely enough to be less flexible in an aqueous solution. Therefore, a moderate length was useful for preparation of aqueous stable self-aggregates and the above 12-atom spacer was effective in the present systems.

4. Concluding remarks

Chlorophyll–galactose conjugates were readily prepared by EDC–HOBt coupling of carboxylated chlorophylls with unprotected galactosides possessing an amino terminal. Such synthetic compounds **11**, **15**, and **16** were more hydrophilic than the corresponding molecule lacking a galactosyl moiety as in **1**, and some of them (**11d** and **16**) self-aggregated in a well-ordered fashion to give chlorosomal J-aggregates in an aqueous solution. The hydrophilic chlorophylls are promising as photosensitizing agents of PDT (see also Section 1). The specific interaction of galactosyl residues with a lectin [1,40] would also be advantageous for their utilization in PDT including target of cancer cells.

Zinc bacteriochlorophyll-*d* analogs **17** possessing an oligo(ethylene glycol) moiety could not give stable and large J-aggregates in an aqueous solution due to the presence of their terminal hydroxy group [2,4]. In contrast, an unprotected galactosyl group did not disturb the formation of chlorosomal J-aggregates. Four hydroxy groups in the galactosyl group interacted with each other intramolecularly and did not compete with the 3^1-hydroxy group, which is an important chromophore to prepare well-ordered J-aggregates. Any glycoside moieties are useful for introduction of hydrophilicity to such supramolecules.

Acknowledgements

We thank Mrs. Masanori Matsumoto and Shohei Kosaka of Ritsumeikan University for their assistance in the experiments and Dr. Tomohiro Miyatake of Ryukoku University for useful discussion. This work was partially supported by Grant-in-Aid for Scientific Research (B) (No. 19350088) from the Japan Society for the Promotion of Science (JSPS) as well as by the "Academic Frontier" Project for Private Universities: matching fund subsidy from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government, 2003–2007.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2009.01.008.

References

[1] X. Zheng, R.K. Pandey, Anti-Cancer Agents Med. Chem. 8 (2008) 241–268.
[2] T. Miyatake, H. Tamiaki, H. Shinoda, M. Fujiwara, T. Matsushita, Tetrahedron 58 (2002) 9989–10000.
[3] V. Huber, M. Katterle, M. Lysetska, F. Würthner, Angew. Chem. Int. Ed. 44 (2005) 3147–3151.
[4] T. Miyatake, S. Tanigawa, S. Kato, H. Tamiaki, Tetrahedron Lett. 48 (2007) 2251–2254.
[5] H. Tamiaki, R. Shibata, T. Mizoguchi, Photochem. Photobiol. 83 (2007) 152–162.
[6] S. Mansouri, S. Fery-Forgues, B. Meunier, N. Paillous, J. Chem. Soc., Perkin Trans. 2 (1996) 1649–1654.
[7] I. Eichwurzel, H. Stiel, K. Teuchner, D. Leupold, H. Scheer, Y. Salomon, A. Scherz, Photochem. Photobiol. 72 (2000) 204–209.
[8] H. Taima, A. Okubo, N. Yoshioka, H. Inoue, Chem. Eur. J. 12 (2006) 6331–6340.
[9] K.E. Borbas, V. Chandrashaker, C. Muthiah, H.L. Kee, D. Holten, J.S. Lindsey, J. Org. Chem. 73 (2008) 3145–3158.
[10] R.E. Fenna, B.W. Matthews, Nature 258 (1975) 573–577.
[11] E. Hofmsnn, P.M. Wrench, F.P. Sharples, R.G. Hiller, W. Welte, K. Diederichs, Science 272 (1996) 1788–1791.
[12] H. Hirabayashi, M. Amakawa, Y. Kamimura, Y. Shino, H. Satoh, S. Itoh, H. Tamiaki, J. Photochem. Photobiol. A: Chem. 183 (2006) 121–125.
[13] D. Horigome, H. Satoh, N. Itoh, K. Mitsunaga, I. Oonishi, A. Nakagawa, A. Uchida, J. Biol. Chem. 282 (2007) 6525–6531.
[14] N.-U. Frigaard, A.G.M. Chew, H. Li, J.A. Maresca, D.A. Bryant, Photosynth. Res. 78 (2003) 93–117.
[15] J.M. Linnanto, J.E.I. Korppi-Tommola, Photosynth. Res. 96 (2008) 227–245.
[16] Y. Saga, Y. Shibata, S. Itoh, H. Tamiaki, J. Phys. Chem. B 111 (2007) 12605–12609.
[17] P.G. Sørensen, R.P. Cox, M. Miller, Photosynth. Res. 95 (2008) 191–196.
[18] K. Uehara, M. Mimuro, Y. Ozaki, J.M. Olson, Photosynth. Res. 41 (1994) 235–243.
[19] P.I. van Noort, Y. Zhu, R. LoBrutto, R.E. Blankenship, Biophys. J. 72 (1997) 316–325.
[20] D.B. Steensgaard, H. Wackerbarth, P. Hildebrandt, A.R. Holzwarth, J. Phys. Chem. B 104 (2000) 10379–10386.
[21] B. Zietz, V.I. Prokhorenko, A.R. Holzwarth, T. Gillbro, J. Phys. Chem. B 110 (2006) 1388–1393.
[22] R. Shibata, H. Tamiaki, Bioorg. Med. Chem. 14 (2006) 2235–2241.
[23] H. Tamiaki, M. Amakawa, Y. Shimono, R. Tanikaga, A.R. Holzwarth, K. Schaffner, Photochem. Photobiol. 63 (1996) 92–99.
[24] C.M. Jakobsen, S.R. Denmeade, J.T. Isaacs, A. Gady, C.E. Olsen, S.B. Christensen, J. Med. Chem. 44 (2001) 4696–4703.
[25] J. Sirieix, N.L. Viguerie, M. Riviére, A. Lattes, New J. Chem. 24 (2000) 1043–1048.
[26] H. Tamiaki, JP Patent 2004-262781 (2004).
[27] B.G. Davis, M.A.T. Maughan, M.P. Green, A. Ulman, J.B. Jones, Tetrahedron Asymm. 11 (2000) 245–262.
[28] A. Sasaki, N. Murahashi, H. Yamada, A. Morikawa, Biol. Pharm. Bull. 18 (1995) 740–746.
[29] H. Cheng, X. Cao, M. Xian, L. Fang, T.B. Cai, J.J. Ji, J.B. Tunac, D. Sun, P.G. Wang, J. Med. Chem. 48 (2005) 645–652.
[30] H. Susaki, K. Suzuki, M. Ikeda, H. Yamada, H.K. Watanabe, Chem. Pharm. Bull. 42 (1994) 2090–2096.
[31] D. Michalik, J.F.G. Viegenthart, J.P. Kamerling, J. Chem. Soc., Perkin Trans. 1 (2002) 1973–1981.
[32] M. Sakagami, K. Horie, K. Higashi, H. Yamada, H. Hamana, Chem. Pharm. Bull. 47 (1999) 1237–1245.
[33] P.H. Weigel, M. Nao, S. Roseman, Y.C. Lee, Carbohydrate Res. 70 (1979) 83–91.
[34] H. Uzawa, H. Ito, M. Izumi, H. Tokuhisa, K. Taguchi, N. Minoura, Tetrahedron 61 (2005) 5895–5905.
[35] S. Orlandi, R. Annunziata, M. Benaglia, F. Cozzi, L. Manzoni, Tetrahedron 61 (2005) 10048–10060.
[36] Y. Mikata, Y. Shinohara, K. Yoneda, Y. Nakamura, K. Esaki, M. Tanahashi, I. Brudziñska, S. Hirohara, M. Yokoyama, K. Mogami, T. Tanase, T. Kitayama, K. Takashiba, K. Nabeshima, R. Takagi, M. Takatani, T. Okamoto, I. Kinoshita, M. Doe, A. Hamazawa, M. Morita, F. Nishida, T. Sakakibara, C. Orvig, S. Yano, J. Org. Chem. 66 (2001) 3783–3789.
[37] H. Tamiaki, T. Michitsuji, R. Shibata, Photochem. Photobiol. Sci. 7 (2008) 1225–1230.
[38] T. Miyatake, H. Tamiaki, A.R. Holzwarth, K. Schaffner, Helv. Chim. Acta 82 (1999) 797–810.
[39] T. Miyatake, H. Tamiaki, J. Photochem. Photobiol. C: Photochem. Rev. 6 (2005) 89–107.
[40] H. Tamiaki, Y. Azefu, R. Shibata, R. Sato, K. Toma, Colloids Surf. B: Biointerf. 53 (2006) 87–93.