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SYNTHESIS OF 3-PYRAZOLINYL CHLORINS RELATED TO CHLOROPHYLL BY 1,3-DIPOLAR CYCLOADDITION REACTION FROM METHYL PHEOPHORBIDE-*a*

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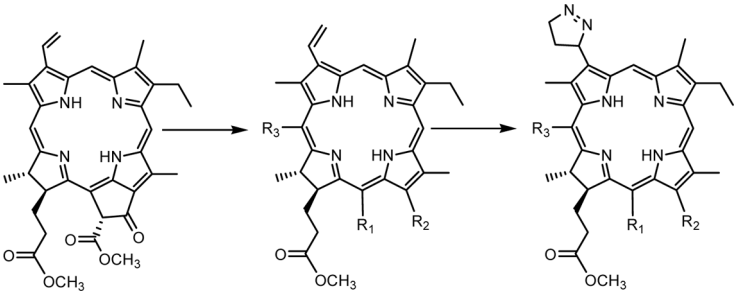
GRAPHICAL ABSTRACT



Abstract *The synthesis of a series of 3-pyrazolinyl-substituted chlorins, possessing different basic skeletons of chlorophyll degradation products such as pheophorbide-*a*, pyropheophorbide-*a*, purpurin-18, purpurin-7, purpurin-5, chlorin-p_6, and chlorin-e_6, was fulfilled from methyl pheophorbide-*a* by chemical modification and 1,3-dipolar cycloaddition with diazomethane. The structures of new chlorins were characterized by ultraviolet (UV), infrared (IR), ^1H NMR, and mass spectra and elemental analysis.*

Keywords Chemical modification; chlorophyll-*a* derivative; 1,3-dipolar cyclicaddition; methyl pheophorbide-*a*; photodynamic therapy (PDT)

INTRODUCTION

Photodynamic therapy (PDT) is an experimental cancer treatment modality that selectively destroys cancer cells by interaction of light with a photosensitizing dye, presumably due to the formation of singlet oxygen.[1] In continuing efforts to develop new photosensitizers for photodynamic therapy, the design and synthesis of chlorin derivatives having high selectivity for removal of tumor cells from healthy cells are important challenges in the PDT field. In these earlier works, chlorophyll and its derivatives have been the preferred precursor for the synthesis of new photosensitizers

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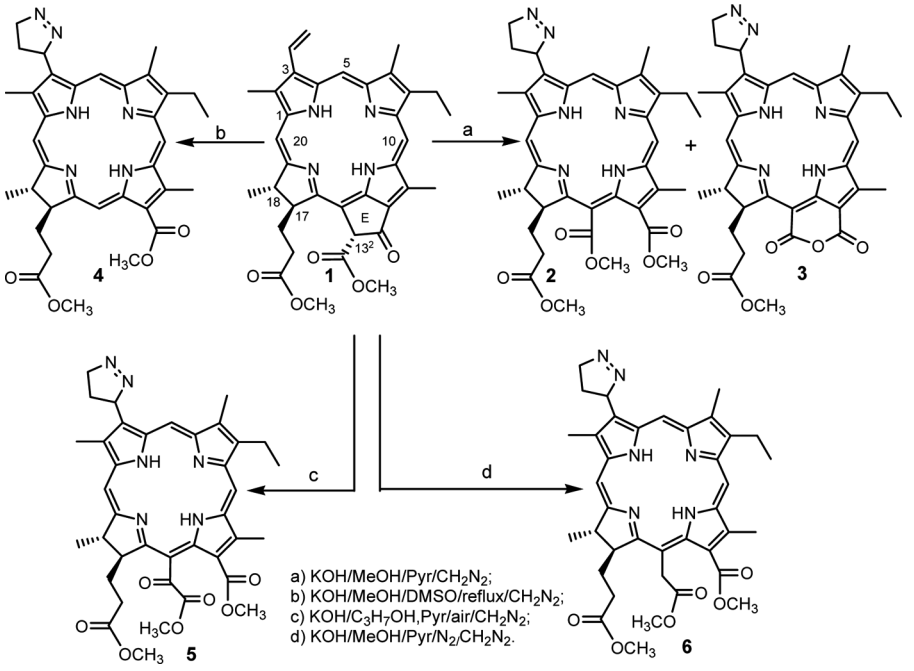
used in PDT. A variety of photosensitizers related to natural chlorins have been synthesized and evaluated for PDT efficiency.[2–6] Many naturally occurring (bacterio) chlorophylls have a vinyl group attached directly to their chlorin *p*-systems at the 3-position such as the most common chlorophyll-*a*. This landmark structure was widely utilized for the modifications and reconstructions of chlorins related to chlorophyll in which almost all chemical reactions of alkene were included. The relevant research of quantitative structure–activity relationship (QSAR) showed that different C3-functional groups of chlorins profoundly influenced their effects on PDT. Recently, chlorophyll derivatives containing heterocyclic substituents at the 3-position have received a great deal of attention because these heterocyclic components characterize their structural, spectroscopic, and potential biological properties. Several kinds of C3-heterocycle substituted chlorophyll-*a* derivatives, such as thienyl, triazolyl, indoyl, pyrazolyl, and quinazolyl groups, have also been synthesized, among which C3-pyrazolyl-substituted chlorins show excellent anticancer properties in photodynamic therapy.[7–9] To investigate systematically C3-heterocycle-substituted chlorins and search for effective photosensitizers for use in PDT, the synthesis of novel 3-pyrazolylchlorins with a basic carbon frame of chlorophyll-*a* was completed in the present work.

RESULTS AND DISCUSSION

Methyl pheophorbide-*a* (**1**, MP*a*), as starting material separated from *Spirulina maxima* alga, was treated with methanolic KOH in the presence of pyridine under an inert atmosphere for 50 min followed by acidification with AcOH and methylation and 1,3-dipolar cycloaddition with excess ethereal diazomethane for 24 h to give 3-pyrazolinylchlorin-p_6 trimethyl ester (**2**) in 27% yield and 3-pyrazolinylpurpurin-18 methyl ester (**3**) in 36% yield, respectively. The 1,3-dipolar cycloaddition of purpurin-5 dimethyl ester was carried out by an improved method, in which MPa (**1**) was refluxed in the mixed solution of methanol and dimethylsulfoxide (DMSO) containing KOH in the open and treated with CH_2N_2 for 48 h after acidification with AcOH to give 57% of C3-pyrazolinyl-substituted purpurin-5 dimethyl ester (**4**). The pheophorbide-*a* (**1**) was oxidized in propyl alcohol containing KOH in the presence of pyridine by bubbling air for 2 h, acidizing it with concentrated HCl, and treating it with excess ethereal diazomethane for 24 h to offer 3-pyrazolinylpurpurin-7 trimethyl ester (**5**) in 56% yield. These successive reactions included allomerization and rearrangement of keto-ester on the exocyclic ring and 1,3-dipolar cycloaddition at the 3-position.[10] 3-Pyrazolinylchlorin-e_6 trimethyl ester (**6**) was prepared in 50% yield by stirring in the mixed solution of methanol and pyridine containing KOH under a nitrogen atomosphere and treatment with CH_2N_2 for 60 h after acidification. These chlorins (**2**–**6**) were synthesized without separation from starting material **1** combining the functional group conversion on the *E*-ring and the cycloaddition with diazomethane (Scheme 1).

The decarbomethoxylation of MP*a* (**1**) was carried out by refluxing in acetic acid to give methyl pyropheophorbide-*a* (**7**, MPP*a*). Its *E*-cycloketone moiety was reduced with $NaBH_4$ in MeOH in the presence of trifluoroacetic acid to produce 13^2-deoxo-MPPa (**8**) in yields of 56%. 20-Chlorochlorin (**9**) was obtained in 85% yield from the starting material **7** by electrophilic substitution using N-chlorosuccinimide (NCS)



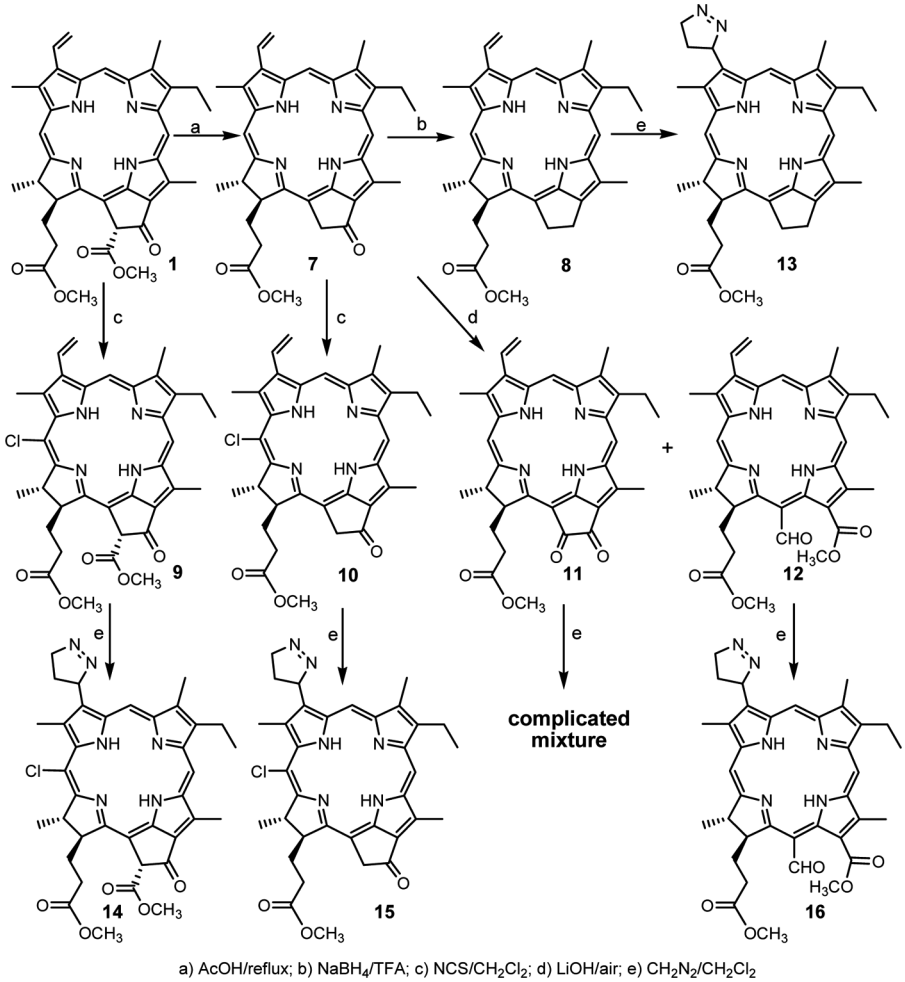
Scheme 1. Synthesis of C3-pyrazolylchlorins from pheophorbide-*a* by one-pot method.

as chlorinating agent in dichloromethane. The chlorination of pyroheophorbide-*a* (**7**) was completed by the same method to afford chloro-substituted chlorin (**10**) in 80% yield. MPPa (**7**) was stirred in saturated methanol with LiOH by bubbling air for 3 h and treated with AcOH and CH_2N_2 for acidification and methylation to give 13^2-oxopyro-pheophorbide-*a* (**11**, 32%) and purpurin-5 dimethyl esters (**12**, 15%) as the main products.

The 1,3-dipolar cycloadditions of chlorins (**8**–**10**) with excess diazomethane were performed to generate smoothly C3-pyrazolinyl-substituted chlorins (**13**–**15**) in different yields and reaction times. The corresponding cyclization of compound **11** with diazomethane was ot successful because the α-diketon moiety on the *E*-ring participated in a reaction to form a very complicated mixture. The chlorin (**12**) was treated with ethereal diazomethane to give 3-pyrazolinyl purpurin-5 dimethyl esters (**16**) in 58% yield (Scheme 2).

The structures of all pyrazolyl-substituted chlorins indicate that the 1,3-dipolar cycloaddition with diazomethane has a particularly regioselective preference to produce anti-Markovniko-type products; namely, the positive dipole end of diazomethane links to the carbon atom at the 3a-position, and the negative dipole end connects with the C3b-carbon. All pyrazolyl-chlorins are a mixture of two (*R*/*S*)-epimers in an approximate ratio of 1:1 due to the chiral center at the 3a-carbon atom. These isomeric mixtures were clearly evident in their ^{1}H NMR spectrum, showing splitting of the resonances for the adjacent protons such as 5-*meso*-H, C2-methyl group or other functional group.

It was found that the rates and yields for the 1,3-dipolar cycliaddition of chlorophyll-*a* degradation products depended on the electron-withdrawing groups



Scheme 2. Synthesis of C3-pyrazolylchlorins from (pyro)pheophorbide-*a* by stepwise reaction.

linked in the periphery of chlorin chromophore. The more the electron-withdrawing group linked with the terminal of C-D ring of chlorin, the faster the reaction rate became with diazomethane. Comparatively speaking, the formation of chlorin (**13**) in 40% yield, without any carbonyl group at 13- and 15- positions, required 4 days. In contrast, the reaction time for purpurin (**13**), having a methoxycarbonyl group at the 13-position and a formyl group at the 15-position, with CH_2N_2 needed just 24 h to give 58% of cycloadduct (**16**).

CONCLUSIONS

An efficient methodology has been developed for the regioselective synthesis of C3-pyrazolyl-substituted chlorins, related to chlorophyll-*a*, from methyl pheophorbide-*a* by the 1,3-dipolar cycloaddition with diazomethane in one-pot synthesis or

stepwise synthesis. The synthetic methodology utilizing 1,3-cycloaddition of vinyl-substituted chlorins with diazomethane lays the groundwork for important peripheral functionalization of tetrapyrrolic ring systems. The pyrazoline ring at the 3-position can be used as a versatile building block that allows for subsequent transformations into other special structures such as cyclopropane moiety. Such reactions construct convenient five-membered heterocycles on the macrocycles, and these modifications for the parent ring of chlorophyll-*a* derivatives may be valuable in the generation of novel photosensitisers for PDT.

EXPERIMENTAL

The infrared (IR) spectra were measured with a Shimadzu Fourier transform (FT) IR 8300 spectrophotometer. The ultraviolet–visible (UV-vis) spectra were taken with a Unicam SP 800 spectrophotometer. The ^{1}H NMR spectra were recorded with a Varian 400 spectrometer. Mass spectra were recorded by JMX-DX300 in eV. The elemental analyses were performed on a Perkin-Elmer 240 microanalyzer. Melting points (mp) were determined with a WRS-1B melting-point apparatus and are uncorrected. Methyl pheophorbide-*a* (**1**) was obtained according to Smith's method.[11] Chlorine **8** and **10** were synthesized according to Ref. 12. Chlorine **9** was synthesized according to Ref. 13. Chlorine **11** and **12** were synthesized according to Ref. 10.

3-Devinyl-3-[3′(*R*/*S*)-(1′-pyrazolinyl)]-chlorin-p_6 Trimethyl Ester (2) and 3-Devinyl-3-[3′(*R*/*S*)-(1′-pyrazolinyl)]-purpurin-18 Methyl Ester (3)

Methyl pheophorbide-*a* **1** (160 mg, 0.264 mmol) was dissolved in 5 mL of pyridine. To this solution, 80 mL of saturated methanol with KOH were added, and the mixture was stirred in an open system in the dark for 2 h, poured into cool water, adjusted pH to 3 with hydrochloric acid, and then extracted with dichloromethane (2×50 mL). The combined extract was washed with water, dried over anhydrous Na_2SO_4, and concentrated to 10 mL. The resulting solution was treated with excess ethereal diazomethane, stirred in the dark for 24 h and with 25% acetic acid (10 mL) to remove unreacted CH_2N_2. The mixture was poured into water (30 mL) and extracted with dichloromethane (20 mL × 2). The extract was washed with water (20 mL × 2) and dried over anhydrous Na_2SO_4. The evaporation residue was purified by using chromatography on a silica-gel column with hexane–ethyl acetate (4:1) to give **2** as a green solid (48 mg, 27%) and **3** as a red solid (59 mg, 36%), respectively. compound **2**: mp: 225–228 °C; UV-vis ($CHCl_3$) λ_{max}: 405 (relative intensity, 1.00), 500 (0.12), 534 (0.05), 606 (0.06), 661 (0.38) nm; ^{1}H NMR ($CDCl_3$) δ: −1.03, 0.33 (each br s, each 1H, NH), 1.68 (t, $J = 7.6$ Hz, 3H, 8-CH_3), 1.82 (d, $J = 7.4$ Hz, 3H, 18-CH_3), 2.00–2.82 (each m, 6H, 4′-H + 17a + 17b-H), 3.72 (q, $J = 7.6$ Hz, 2H, 8a-H), 4.23, 4.17, 3.91, 3.52, 3.27 (3.26), 3.18 (each s, each 3H or 1.5H, $OCH_3 + CH_3$), 4.42 (q, $J = 7.0$ Hz, 1H, 18-H), 5.17 (d, $J = 8.6$ Hz, 1H, 17-H), 5.39–5.50, 4.67–4.82 (each m, each 1H, 5′-H), 6.68 (t, $J = 8.6$ Hz, 1H, 3′-H), 9.71, 9.11, 8.70 (each s, each 1H, *meso*-H). IR (KBr) *v*: 3456 (N-H), 2960 (C-H), 1743, 1726, 1703 (C=O), 1608 (C=C), 1535 (chlorin skeleton) cm^{-1}. MS (70 eV) *m*/*z* (%): 667.3 ($M^+ + 1$, 100). Anal. calcd. for $C_{37}H_{42}N_6O_6$: C, 66.65; H, 6.35; N, 12.60. Found: C, 66.46; H, 6.48; N, 12.77. Compound **3**: mp: 203–205 °C; UV-vis ($CHCl_3$) λ_{max}: 410 (relative intensity,

1.00), 477 (0.04), 506 (0.06), 542 (0.17), 642 (0.07), 696 (0.36) nm; ^{1}H NMR ($CDCl_3$) δ: −1.74, 0.42 (each br s, each 1H, NH), 1.55 (t, $J = 7.6$ Hz, 3H, 8-CH_3), 1.76 (d, $J = 7.4$ Hz, 3H, 18-CH_3), 1.98–2.18 (m, 1H, 4′-H), 2.20–2.15, 2.37–2.57 (each m, each 2H, 17a + 17b-H), 2.71–2.84(m, 1H, 4′-H), 3.55 (q, $J = 7.6$ Hz, 2H, 8a-H), 3.06, 3.23 (3.22), 3.52 (3.54), 3.61 (3.62) (each s, each 3H or 1.5H, OCH_3+CH_3), 4.35–4.45 (m, 1H, 18-H), 4.77 (td, $J = 18.0$, 8.9 Hz, 1H, 5′-H), 5.14 (d, $J = 8.6$ Hz, 1H, 17-H), 5.48 (dd, $J = 18.0$, 9.8 Hz, 1H, 5′-H), 6.57 (t, $J = 7.8$ Hz, 1H, 3′-H), 8.60, 8.95 (8.98), 9.25 (each s, each 1H or 1.5H, *meso*-H). IR (KBr) *v*: 3440 (N-H), 2975–2850, 1749 (C=O), 1639 (C=N), 1617 (C=C), 1552 (chlorin skeleton) cm^{-1}. MS (70 eV) *m/z* (%): 621.4 ($M^+ + 1$, 100). Anal. calcd. for $C_{35}H_{36}N_6O_5$: C, 67.73; H, 5.85; N, 13.54. Found: C, 67.59; H, 6.08; N, 13.71.

3-Devinyl-3-[3′(*R/S*)-(1′-pyrazolinyl)]-purpurin-5 Dimethyl Ester (4)

Methyl pheophorbide-*a* **1** (168 mg, 0.277 mmol) was dissolved in methanol saturated with KOH (10 mL) and DMSO (15 mL) and stirred in an open system in the dark for 6 h. The resulting mixture was refluxed for 5 h, poured into cool water, and extracted with dichloromethane (2 × 50 mL) after adjusting pH to 4 with hydrochloric acid. The combined extract was washed with water, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude product was redissolved in CH_2Cl_2 (5 mL), treated with excess ethereal diazomethane, and stirred in the dark for 48 h. After adding acetic acid (5 mL) to remove unreacted CH_2N_2, the mixture was evaporated under vacuum. The residue was chromatographed on a silica-gel column with hexane–ethyl cetate (3:1) to give **4** (89 mg, 53%) as a green solid. Mp: 221–223 °C; UV-vis ($CHCl_3$) λ_{max}: 399 (relative intensity, 1.00), 497 (0.11), 524 (0.06), 606 (0.05), 660 (0.48) nm; ^{1}H NMR ($CDCl_3$) δ: 0.06, 0.86 (each br s, each 1H, NH), 1.71 (t, $J = 7.5$ Hz, 3H, 8-CH_3), 1.88 (d, $J = 7.1$ Hz, 3H, 18-CH_3), 2.07–2.28, 2.32–2.52, 2.55–2.65 (each m, 4H, 17a + 17b-H), 2.68–2.82 (m, 2H, 4′-H), 3.77 (q, $J = 7.5$ Hz, 2H, 8a-H), 3.31, 3.25, 3.62, 3.82, 4.35 (each s, each 3H, $OCH_3 + CH_3$), 4.46–4.56 (m, 2H, 17-H + 18-H), 4.77 (dtd, $J = 17.9$, 9.8, 2.5 Hz, 1H, 5′-H), 5.14 (d, $J = 8.6$ Hz, 1H, 17-H), 5.48 (dd, $J = 17.9$, 9.8 Hz, 1H, 5′-H), 6.75 (t, $J = 9.8$ Hz, 1H, 3′-H), 8.77, 9.23, 9.77, 9.84 (each s, each 1H, *meso*-H). IR (KBr) *v*: 3446 (N-H), 1741, 1710 (C=O), 1612 (C=C), 1533 (chlorin skeleton) cm^{-1}. MS (70 eV) *m/z* (%): 609.4 ($M^+ + 1$, 100). Anal. calcd. for $C_{35}H_{40}N_6O_4$: C, 69.06; H, 6.62; N, 13.81. Found: C, 69.27; H, 6.76; N, 13.60.

3-Devinyl-3-[3′(*R/S*)-(1′-pyrazolinyl)]-purpurin-7 Trimethyl Ester (5)

A KOH solution in *n*-propanol (3 g dissolved in 20 mL) was added to a solution of **1** (158 mg, 0.260 mmol) in a mixed solution of ether (100 mL) and pyridine (5 mL). The mixture was stirred at room temperature by bubbling air for 2 h, acidized by 2% aqueous HCl and extracted with CH_2N_2 (5 × 30 mL). The combined extract was washed with 4% aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude product was redissolved in CH_2Cl_2 (5 mL), treated with excess ethereal diazomethane, and stirred in the dark for 24 h. The resulting mixture was treated with acedic acid for moving unreacted CH_2N_2, and evaporated to dry. The residue was chromatographed on silica gel by silica-gel chromatography with

hexane–ethyl acetate (4:1) to give **5** (105 mg, 58%) as a red-brown solid. Mp: 199–202 °C; UV-vis ($CHCl_3$) λ_{max}: 402 (relative intensity, 1.00), 500 (0.09), 538 (0.08), 632 (0.06), 669 (0.27) nm; ^{1}H NMR ($CDCl_3$) δ: −0.25, −0.15 (each br s, each 1H, NH), 1.66 (t, $J = 7.6$ Hz, 3H, 8-CH_3), 1.79 (d, $J = 7.3$ Hz, 3H, 18-CH_3), 1.71–1.83, 2.01–2.14 (each m, each 2H, 17a + 17b-H), 2.32–2.42 (m, 1H, 4′-H), 2.71–2.82 (m, 1H, 4′-H), 3.67 (q, $J = 7.6$ Hz, 2H, 8a-H), 3.12, 3.21, 3.53, 3.61, 4.15 (each s, each 3H, $OCH_3 + CH_3$), 4.33 (q, $J = 7.2$ Hz, 1H, 18-H), 4.68 (d, $J = 8.5$ Hz, 1H, 17-H), 4.76 (ddd, $J = 18.0$, 9.8, 2.6 Hz, 1H, 5′-H), 5.46 (ddt, $J = 18.0$, 9.8, 2.6 Hz, 1H, 5′-H), 6.58 (t, $J = 9.7$ Hz, 1H, 3′-H), 8.53, 8.95, 9.62 (each s, each 1H, *meso*-H). IR (KBr) *v*: 3334 (N-H), 2850 (C-H), 1733, 1708, 1703 (C=O), 1616 (C=C), 1585 (chlorin skeleton) cm^{-1}. MS (70 eV) m/z (%): 695.3 ($M^+ + 1$, 100). Anal. calcd. for $C_{39}H_{42}N_6O_7$: C, 65.69; H, 6.09; N, 12.10; Found: C, 65.47; H, 6.10; N, 12.23.

3-Devinyl-3-[3′(*R*/*S*)-(1′-pyrazolinyl)]-chlorin-e_6 Trimethyl Ester (6)

Methanolic potassium hydroxide (5%, 100 mL) was added to a solution of methyl pheophorbide-*a* **1** (120 mg, 0.198 mmol) in pyridine (10 mL), and the mixture was stirred at room temperature in the dark for 30 min. It was then diluted with water (~200 mL), neutralized with acetic acid, and extracted with dichloromethane (2 × 50 mL). The combined organic extract was shaken with 5% hydrochloric acid (3 × 100 mL), washed with water, dried over anhydrous Na_2SO_4, and concentrated to dryness. The crude product was treated with excess ethereal diazomethane and stirred in the dark for 60 h. After adding acetic acid (5 mL) to remove unreacted CH_2N_2, the mixture was evaporated under vacuum. The residue was chromatographed on a silica-gel column with hexane–ethyl acetate (3:1) to give **6** (67 mg, 50%) as a green solid. Mp: 200–203 °C; UV-vis ($CHCl_3$) λ_{max}: 407 (relative intensity, 1.00), 500 (0.32), 534 (0.05), 606 (0.06), 661 (0.38) nm; ^{1}H NMR ($CDCl_3$) δ: −1.59, −1.33 (each br s, each 1H, NH), 1.70 (t, $J = 7.6$ Hz, 3H, 8-CH_3), 1.75 (d, $J = 7.1$ Hz, 3H, 18-CH_3), 2.05–2.15 (m, 1H, 4′-H), 2.16–2.24, 2.52–2.62 (each m, each 2H, 17a + 17b-H), 2.73–2.83 (m, 1H, 4′-H), 3.87 (q, $J = 7.6$ Hz, 2H, 8a-H), 3.23, 3.32 (3.30), 3.58, 3.63, 3.77, 4.26 (each s, each 3H or 1.5H, OCH_3+CH_3), 4.22–4.43 (m, 1H, 18-H), 4.38–4.52 (m, 1H, 17-H), 4.62–4.82 (m, 1H, 5′-H), 5.42–5.52 (m, 1H, 5′-H), 6.73 (t, $J = 9.6$ Hz, 1H, 3′-H), 8.77, 9.18 (9.16), 9.70 (each s, each 1H or 0.5H, *meso*-H). IR (KBr) *v*: 3344 (N-H), 2862, 2927 (C-H), 1749, 1747, 1703 (C=O), 1629 (C=C), 1591 (chlorin skeleton) cm^{-1}. MS (70 eV) m/z (%): 681.4 ($M^+ + 1$, 100). Anal. calcd. for $C_{38}H_{44}N_6O_6$: C, 67.04; H, 6.51; N, 12.34; Found: C, 67.21; H, 6.38; N, 12.39.

3-Devinyl-13^2-deoxo-3-[3′(*R*/*S*)-(1′-pyrazolinyl)]-pyropheophorbide-*a* Methyl Ester (13)

The excess ethereal diazomethane (approximately 15 mL) was added To dichloromethane (2 mL) in **8** (55 mg, 0.103 mmol) and stirred at room temperature in the dark for 96 h. The reaction mixture was treated with acetic acid to remove unreacted CH_2N_2, poured into water, and extracted with CH_2Cl_2. After evaporation in vacuo, the residue was purified by chromatography on a silica-gel column with hexane–ethyl acetate (5:1) to give **13** (24 mg, 40%) as a green solid.

Mp: 221–223 °C; UV-vis ($CHCl_3$) λ_{max}: 396 (relative intensity, 1.00), 500 (0.10), 588 (0.05), 642 (0.30) nm; ^{1}H NMR ($CDCl_3$) δ: −3.37 (br s, 1H, NH), −1.66 (br s, 1H, NH), 1.74 (t, 3H, $J = 7.6$ Hz, 8a-CH_3), 1.85 (d, 3H, $J = 7.4$ Hz, 18-CH_3), 2.12–2.25, 2.27–2.41, 2.55–2.65, 2.72–2.85 (each m, 6H, 4′-H + 17a+17b-CH_2), 3.38, 3.40, 3.49, 3.57 (each s, each 3H or 1.5H, OCH_3+CH_3), 3.85(q, $J = 7.5$ Hz, 2H, 8a-CH_2), 4.02 (ddd, $J = 17.0$, 6.9, 2.8 Hz, 1H, 15a-H), 4.08 (ddd, $J = 17.0$, 6.9, 3.1 Hz, 1H, 15a-H), 4.49 (dt, $J = 8.8$, 2.2 Hz, 1H, 17-H), 4.68 (q, $J = 7.2$ Hz, 1H, 18-H), 4.77 (ddd, $J = 16.2$, 6.9, 3.1 Hz, 1H, 13a-H), 4.81 (ddd, $J = 16.2$, 6.9, 2.8 Hz, 1H, 13a-H), 4.93 (ddd, $J = 17.0$, 9.6, 3.0 Hz, 1H, 5′-H), 5.49 (ddt, $J = 17.0$, 9.6, 2.6 Hz, 1H, 5′-H), 6.93(t, $J = 9.8$ Hz, 1H, 3′-H), 8.96, 9.54 (9.52), 9.59 (each s, each 1H or 0.5H, *meso*-H). IR (KBr) v: 3446 (N-H), 2960, 2927 (C-H), 1737 (C=O), 1674 (C=C), 1542 (chlorin skeleton). MS (70 eV) m/z (%): 577.4 ($M^+ + 1$, 100). Anal. calcd. for $C_{35}H_{40}N_6O_2$: C, 72.89; H, 6.99; N, 14.57. Found: C, 72.70; H, 6.77; N, 14.51.

3-Devinyl-3-[3′(*R*/*S*)-(1′-pyrazolinyl)]-20-chloropheophorbide-*a* Methyl Ester (14)

This compound was obtained as a red solid from compound **9** by reaction with CH_2N_2 for 30 h in the yield of 56% according to the method for preparing compound **13**. UV-vis ($CHCl_3$) λ_{max}: 414 (relative intensity, 1.00), 518 (0.10), 550 (0.14), 620 (0.07), 678 (0.46) nm; ^{1}H NMR ($CDCl_3$) δ: −2.01 (br s, 1H, NH), −1.96 (br s, 1H, NH), 1.64 (1.63) (d, 3H, $J = 7.0$ Hz, 18-CH_3), 1.66 (t, 3H, $J = 7.6$ Hz, 8a-CH_3), 2.02–2.35, 2.40–2.66 (each m, 5H, 4′-H + 17a+17b-CH_2), 2.73–2.84 (m, 1H, 4′-H), 3.15 (3.17), 3.49 (3.50), 3.54 (3.55), 3.68, 3.89 (3.90) (each s, each 3H or 1.5H, OCH_3+CH_3), 3.65 (q, $J = 7.6$ Hz, 2H, 8a-CH_2), 4.10–4.18 (m, 1H, 17-H), 4.22–4.30 (m, 1H, 18-H), 4.72–4.86 (m, 1H, 5′-H), 5.50–5.61 (m, 1H, 5′-H), 6.28 (6.25) (each s, each 0.5H, 13^2-H), 6.64(t, $J = 9.5$ Hz, 1H, 3′-H), 9.03 (9.02), 9.50 (9.53) (each s, each 0.5H, *meso*-H). IR (KBr) v: 3438 (N-H), 2986, 2870 (C-H), 1733 (C=O), 1610 (C=C), 1539 (chlorin skeleton) cm^{-1}. MS (70 eV) m/z (%): 683.4 ($M^+ + 1$, 100). Anal. calcd. for $C_{37}H_{39}ClN_6O_5$: C, 65.05; H, 5.75; N, 12.30. Found: C, 65.16; H, 5.70; N, 12.56.

3-Devinyl-3-[3′(*R*/*S*)-(1′-pyrazolinyl)]-20-chloropyropheophorbide-*a* Methyl Ester (15)

This compound was obtained as a red solid from compound **10** by reaction with CH_2N_2 for 30 h in the yield of 60% according to the method for preparing compound **13**. UV-vis ($CHCl_3$) λ_{max}: 414 (relative intensity, 1.00), 517 (0.09), 550 (0.14), 621 (0.06), 678 (0.46) nm; ^{1}H NMR ($CDCl_3$) δ: −1.99 (br s, 1H, NH), 0.83 (br s, 1H, NH), 1.61–1.69 (m, 6H, 8a-CH_3 + 18-CH_3), 2.05–2.27, 2.40–2.63 (each m, 5H, 4′-H + 17a+17b-CH_2), 2.75–2.80(m, 1H, 4′-H), 3.11, 3.48, 3.59, 3.60 (each s, each 3H or 1.5H, OCH_3+CH_3), 3.61(q, $J = 7.6$ Hz, 2H, 8a-CH_2), 4.24 (4.23)(d, $J = 8.7$ Hz, 1H, 17-H), 4.76 (dq, $J = 8.9$, 2.5 Hz, 1H, 18-H), 4.78–4.84 (m, 1H, 5′-H), 5.23 (s, 2H, 132-H), 5.49–5.58 (m, 1H, 5′-H), 6.28 (6.25) (each s, each 0.5H, 13^2-H), 6.63 (6.62) (t, $J = 10.1$ Hz, 1H, 3′-H), 9.00(899), 9.46 (each s, each 0.5H, *meso*-H). IR (KBr) *v*: 3350 (N-H), 2964, 2869 (C-H), 1747, 1699 (C=O), 1606 (C=C), 1533 (chlorin skeleton)

cm^{-1}. MS (70 eV) m/z (%): 625.3 ($M^+ + 1$, 100). Anal. calcd. for $C_{35}H_{37}ClN_6O_3$: C, 67.24; H, 5.97; N, 13.44. Found: C, 67.07; H, 6.12; N, 13.53.

3-Devinyl-3-[3′(*R*/*S*)-(1′-pyrazolinyl)]-15-formyl-purpurin-5 Dimethyl Ester (16)

This compound was obtained as a red solid from compound **12** by reaction with CH_2N_2 for 24 h in the yield of 58% according to the method for preparing compound **13**. UV-vis ($CHCl_3$) λ_{max}: 408 (relative intensity, 1.00), 504 (0.07), 538 (0.09), 635 (0.05), 687 (0.30) nm; ^{1}H NMR ($CDCl_3$) δ: −0.32 (br s, 1H, NH), −0.05(br s, 1H, NH), 1.65 (t, $J = 7.6$ Hz, 3H, 18-CH_3), 1.80 (d, $J = 7.2$ Hz, 3H, 8a-CH_3), 1.93–2.10(m, 2H, 4′-H), 2.20–2.28, 2.32–2.42, 2.48–2.59, 2.66–2.79 (each m, 4H, 17a+17b-CH_2), 2.75–2.80(m, 1H, 4′-H), 3.12, 3.20, 3.56, 3.58, 4.19 (each s, each 3H or 1.5H, OCH_3+CH_3), 3.65 (q, $J = 7.6$ Hz, 2H, 8a-CH_2), 4.42 (4.41) (q, $J = 7.1$ Hz, 1H, 18-H), 4.73 (dt, $J = 17.9$, 9.6 Hz, 1H, 5′-H), 5.06 (dt, $J = 9.3$, 2.3 Hz, 1H, 17-H), 5.44 (dd, $J = 17.9$, 9.6 Hz, 1H, 5′-H), 6.28 (6.25) (each s, each 0.5H, 13^2-H), 6.55–6.61 (m, 1H, 3′-H), 8.56, 9.01 (9.02), 9.61 (each s, each 0.5H or 1H, *meso*-H), 11.48 (s, 1H, CHO). IR (KBr) v: 3323 (N-H), 2956, 2850 (C-H), 1735, 1697 (C=O), 1605 (C=C), 1502 (chlorin skeleton) cm^{-1}. MS (70 eV) m/z (%): 637.2 ($M^+ + 1$, 100). Anal. calcd. for $C_{36}H_{40}N_6O_5$: C, 67.91; H 6.33; N, 13.20. Found: C, 67.77; H, 6.18; N, 13.24.

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