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ORIGINAL PAPER

Synthesis of purpurin-18 imide derivatives from chlorophyll-*a* and -*b* by modifications and functionalizations along their peripheries

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Abstract The methyl pheophorbide-*a* and methyl pheophorbide-*b* were used as starting materials and converted to purpurin-18 ester by ring-opening and rearrangement reaction in their exocyclic ring. *N*-Substituted purpurin-18 imides were obtained from purpurin-18 ester through amidation reaction of six-membered cyclic anhydride. Further chemical modifications along their peripheries were carried out by a variety of common reactions, including electrophilic substitution, Wittig reaction, allomerization and Vilsmeier reaction, to afford the title compounds with long-wavelength absorption. The structures of all new chlorins were characterized by elemental analysis, IR, UV–vis and ^{1}H NMR spectra.

Keywords Chlorophyll · Chlorin · Purpurin-18 imide · Chemical modification · Photodynamic therapy (PDT)

Introduction

Synthesis of long wavelength absorbing porphyrins (>700 nm) as ideal photosensitizers used in photodynamic therapy (PDT) has become a hot research area owing to that these candidates can achieve maximum tissue penetration [1–7]. Among such compounds with long-wavelength absorption, some naturally occurring bacteriochlorins have been reported as effective photosensitizers in preliminary in vitro and in vivo studies [8]. However, most of these naturally occurring bacteriochlorins are extremely sensitive to oxidation, which result in rapid transformation into relative stable chlorin state, which has an absorption maximum at or below 670 nm. Furthermore, if a laser is used to excite the bacteriochlorin in vivo, oxidation may result in the formation of a new chromophore absorbing outside the laser window, which reduces the photodynamic efficacy. To render PDT more generally applicable to tumor therapy, long wavelength absorbing photosensitizers, such as stable chlorins are needed, because they should also be able to localized in higher concentration at the tumor site relative to normal tissues under normal physiological conditions.

Chlorophyll-*a* and chlorophyll-*b* are natural chlorin pigments and their degradation products stand in marked contrast to symmetric porphyrin pigments due to substantially stabilized S_1 energies, a strong Qy absorption band, and unique redox reactivites. Therefore, the synthesis of novel photosensitizers with long-wavelength absorption, which possessed the basic skeleton of chlorophyll, has become the focus of research in photodynamic therapy [9–12]. For chlorophyll compounds, the Qy bands as a longest absorption band were strongly affected by the substitutents on the Qy axis (N_{21}–N_{23}), especially structural change of exocyclic rings attached to 13- and 15-positions (see Scheme 1) [13]. The conversions from pheophorbide-*a* to purpurin-18, and to purpurin-18 imide ulteriorly, caused very obvious bathochromic shift in their Qy band whose difference value (ΔQy) was 31 and 6 nm, respectively [14]. Considering that constructing six-membered anhydride or imide moiety on the chromophore of chlorophyll can improve considerably its maximum absorption in UV–V is spectra, the modifications of methyl pheophorbide-*a* **1a** (MPP-*a*) and methyl pheophorbide-*b* **1b** (MPP-*b*), as initial degradative product extracted from *Spirulina pacifica* [15, 16], were carried out to search for potential photosensitizers used in PDT. Based on our early works [17–21], here we report a series of synthesis of purpurin-18 imides with

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the basic skeleton of chlorophyll, which possessed absorption maximum (Qy) above 700 nm by a variety of common reactions.

Experimental

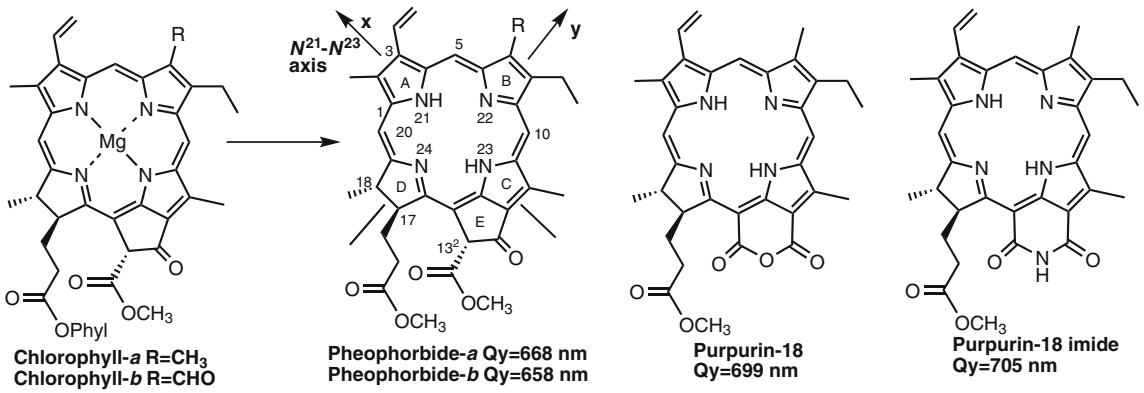
Melting points were determined with an Electrothermal 9100 apparatus and uncorrected. IR spectra were measured with a Shimadzu FT-IR 8300 spectrophotometer. The UV–vis spectra were taken with a Unicam SP 800 spectrophotometer. The ^{1}H NMR spectra were recorded on a Beucker ARX-400 using TMS as internal standard. The elemental analyses were performed on a Perkin-Elmer 240 microanalyer. All chemical reagents were commercially available and purified using standard methods. Solvents were dried in routine ways and redistilled. Methyl pheophorbide-*a* (MPa) **1a** and methyl pheophorbide-*b* (MPa) **1b** were obtained according to Smith's method [15, 16].

Purpurin-18 methyl ester (2)

To a THF solution (25 mL) of **1** (514 mg, 0.847 mmol), an aqueous solution (5 mL) of LiOH (1.2 g) and methanol (15 mL) were sequentially added. This mixture was violently stirred in open system in dark for 3 h, poured into cool water, adjusted to pH 3 with sulfuric acid and then extracted with dichloromethane (2 × 50 mL). The combined extracts were washed with water, dried over anhydrous Na_2SO_4 and treated with CH_2N_2 for short time (approximately 5 min). After evaporation, the residue was purified on chromatography on a silica gel column with hexane–ethyl acetate (5:1) to give 354 mg **2** (0.612 mmol, 70 %) as a red solid. m.p. 275–279 °C; UV–vis ($CHCl_3$) λ_{max}: 410 (relative intensity, 1.00), 479 (0.05), 508 (0.06), 546 (0.18), 699 (0.34) nm; ^{1}H NMR ($CDCl_3$) δ: −0.30 (1H, br s, NH), 0.78 (1H, br s, NH), 1.55 (3H, t, $J = 7.6$ Hz, 8b-CH_3), 1.76 (3H, d, $J = 7.1$ Hz, 18-CH_3), 1.96–2.07, 2.42–2.54, 2.73–2.82 (all 4H, each m, 17a + 17b-H), 3.03, 3.32, 3.56, 3.61 (each 3H, each s, CH_3 + OCH_3), 3.45 (q, 2H, $J = 7.6$ Hz, 8a-CH_2), 4.38–4.49 (1H, m, 18-H), 5.18 (1H, d, $J = 8.2$ Hz, 17-H), 6.15 (1H, d, $J = 11.6$ Hz, *cis*-3b-H), 6.26 (1H, d, $J = 17.8$ Hz, *trans*-3b-H), 7.80 (1H, dd, $J = 17.8$, 11.5 Hz, 3a-H), 8.55, 9.19, 9.25 (each 1H, each s, *meso*-H). The other anlytical data are consistent with the ones in the literature [22].

N-Hydroxylpurpurin-18 imide methyl ester (3a)

The chlorin **2** (75 mg, 0.130 mmol) was dissolved in pyridine (10 mL), to which excess hydroxyl-amine (100 mg) was added with stirring. The resulting solution was stirred at room temperature for 8 h. The reaction mixture was neutralized with concentrated hydrochloric acid by violently stir and extracted with dichloromethane (3 × 100 mL). The combined extracts was washed with saturated $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by chromatography on silica gel column with hexane–ethyl acetate (3:1) to give 63 mg **3a** (0.107 mmol, 82 %) as a red solid. m.p. 258–61 °C; UV–vis ($CHCl_3$) λ_{max}: 421 (relative intensity, 1.00), 484 (0.05), 512 (0.06), 553 (0.19), 661 (0.08), 721 (0.36) nm; ^{1}H NMR ($CDCl_3$) δ: 0.35 (1H, br s, NH), 0.43 (1H, br s, NH), 1.61 (3H, t, $J = 7.6$ Hz, 8b-CH_3), 1.71 (3H, d, $J = 7.3$ Hz, 18- CH_3), 1.90–2.08, 2.37–2.55, 2.73–2.85 (all 4H, each m, 17a + 17b-H), 3.57 (2H, q, $J = 7.6$ Hz, 8a-CH_2), 3.11, 3.28, 3.59, 3.72 (each 3H, each s, OCH_3 + CH_3), 4.28–4.38 (m, 1H, 18-H), 5.25 (br s, 1H, 17-H), 6.17 (1H, d, $J = 11.3$ Hz, *cis*-3b-H), 6.26 (1H, d, $J = 17.4$ Hz, *trans*-3b-H), 7.82 (1H, dd, $J = 17.4$ Hz, 11.3 Hz, 3a-H), 8.49, 9.27, 9.59 (each 1H, each s, *meso*-H). MS *m/z*: 594.3 $(M + H)^+$; IR (KBr) *v*: 3471 (N–H), 2966, 2935 (C–H), 1741–1697 (C=O), 1649 (C=C), 1556 (chlorin skeleton), 1463, 1379, 1174, 1097 956 cm^{-1}; Anal. calcd for



Scheme 1 Structures and Qy absorptions of chlorophyll a and b derivatives

$C_{34}H_{35}N_5O_5$: C 68.79, H 5.94, N 11.80; found C 68.67, H 5.77, N 11.95.

N-Methoxylpurpurin-18 imide methyl ester (3b)

The chlorin **3a** (54 mg, 0.091 mmol) was dissolved in dichloromethane (10 mL), to which excess diazomethane was added in ether (5 mL) with stirring. The resulting solution was stirred at room temperature for 5 min. After adding acetic acid (5 mL) the resultant mixture was poured into water, extracted with dichloromethane (3 × 15 mL). The combined extracts were washed with saturated $NaHCO_3$ and water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by chromatography on silica gel column with hexane–ethyl acetate (3:1) to give 63 mg **3b** (0.087 mmol, 96 %) as a red solid. m.p. 208–211 °C; UV–vis ($CHCl_3$) λ_{max}: 419 (relative intensity, 1.00), 482 (0.05), 514 (0.06), 550 (0.19), 655 (0.08), 709 (0.36) nm; ^{1}H NMR ($CDCl_3$) δ: 0.07 (1H, br s, NH), 0.16 (1H, br s, NH), 1.63 (3H, t, J = 7.6 Hz, 8b-CH_3), 1.72 (3H, d, J = 7.3 Hz, 18-CH_3), 1.90–2.08, 2.40–2.56, 2.73–2.85 (all 4H, each m, 17a + 17b-H), 3.60 (2H, q, J = 7.6 Hz, 8a-CH_2), 3.11, 3.30, 3.59, 3.76 (each 3H, each s, OCH_3 + CH_3), 4.39 (3H, s, 13b-OCH_3), 4.36 (1H, q, J = 7.4 Hz, 18-H), 5.28 (1H, d, J = 9.7 Hz, 17-H), 6.16 (1H, d, J = 11.6 Hz, *cis*-3b-H), 6.25 (1H, d, J = 17.8 Hz, *trans*- 3b-H), 7.86 (1H, dd, J = 17.8 Hz, 11.6 Hz, 3a-H), 8.53, 9.25, 9.51 (each 1H, each s, *meso*-H). The other analytical data consistent with ones in the literature [23].

N-Aminopurpurin-18 imide methyl ester (3c)

The chlorin **2** (200 mg, 0.346 mmol) was dissolved in dichloromethane (50 mL), to which 80 % hydrazine hydrate (0.3 mL) and triethylamine (0.2 mL) were in nitrogen. The resulting solution was stirred at room temperature for 4 h and then poured into iced water and extracted with dichloromethane (3 × 20 mL). The combined extract was dried over anhydrous Na_2SO_4, and the resulting solution was evaporated under vacuum. The residue was chromatographed with a silica gel column (eluent: hexane/ethyl acetate, 3:1) to give 127 mg **3c** (0.215 mmol) as a red solid in 62 % yield. m.p. 235–239 °C; UV–vis ($CHCl_3$) λ_{max}: 417 (relative intensity, 1.00), 504 (0.05), 546 (0.22), 550 (0.21), 713 (0.41) nm; ^{1}H NMR ($CDCl_3$) δ: −1.19 (1H, br s, NH), −0.89 (1H, br s, NH), 1.62 (3H, t, J = 7.4 Hz, 8b-CH_3), 1.81 (3H, d, J = 7.2 Hz, 18-CH_3), 2.08–2.25, 2.34–2.56, 2.60–2.80 (all 4H, each m, 17a + 17b-H), 3.05, 3.31, 3.50, 3.59 (each 3H, each s, CH_3 + OCH_3), 3.62 (q, J = 7.4 Hz, 8a-CH_2), 4.71 (2H, q, J = 7.4 Hz, 18-H), 5.15 (1H, d, J = 9.3 Hz, 17-H), 5.51 (1H, br s, 13b-NH), 6.11 (1H, d, J = 11.4 Hz, *cis*-3b-H), 6.28 (1H, d, J = 18.0 Hz, 1H, *trans*-3b-H), 6.59 (1H, br s, 13b-NH), 7.95 (1H, dd, J = 18.0, 11.4 Hz, 3a-H), 8.88, 9.25, 9.59 (each 1H, each s, *meso*-H); MS *m/z*: 593.3 $(M+H)^+$; IR (KBr) *v*: 3434 (N–H), 2962, 2929 (C–H), 1745–1697 (C=O), 1633 (C=C), 1558 (chlorin skeleton), 1467, 1400, 1313, 1274, 1147, 802 cm^{-1}; Anal. calcd for $C_{34}H_{36}N_6O_4$: C 68.90, H 6.12, N 14.18; found C 69.07, H 6.37, N 14.03.

N-Phenylideneiminepurpurin-18 imide methyl ester (4)

112 mg **3c** (0.189 mmol) was dissolved in dichloromethane (20 mL) to which 0.3 mL of benzaldehyde and catalytic amount of TsOH (10 mg) were added. The resulting mixture was stirred at room temperature for 12 h and poured into water to remain organic layer. After evaporating the residue was chromatographed with a silica gel column (eluent: hexane/ethyl acetate, 3:1) to give 58 mg **4** (0.085 mmol) as a purplish red solid in 45 % yield. m.p. 267–270 °C; UV–vis ($CHCl_3$) λ_{max}: 369 (relative intensity, 0.66), 410 (1.00), 509 (0.11), 550 (0.22), 565 (0.21), 707 (0.41) nm; ^{1}H NMR ($CDCl_3$) δ: 0.22 (1H, br s, NH), 0.29 (1H, br s, NH), 1.65 (3H, t, J = 7.4 Hz, 8b-CH_3),1.71 (3H, d, J = 7.2 Hz, 18-CH_3), 2.39–2.48, 2.67–2.78 (all 4H, each m, 17a + 17b-H), 3.14, 3.32, 3.59, 3.78 (each 3H, each s, CH_3 + OCH_3), 3.62 (q, 2H, J = 7.6 Hz, 8a-H), 4.33 (1H, q, J = 7.2 Hz, 18-H), 5.29 (1H, d, J = 9.2 Hz, 17-H), 6.16 (1H, d, J = 11.5 Hz, *cis*-3b-H), 6.28 (1H, d, J = 18.0 Hz, *trans*-3b-H), 7.54 (2H, d, J = 7.5 Hz, Ph-H), 7.64 (1H, t, J = 7.3 Hz, Ph-H), 7.89 (2H, dd, J = 7.5, 1.3 Hz, Ph-H), 7.87 (1H, dd, J = 17.8, 11.5 Hz, 3a-H), 8.50, 9.29, 9.51 (each 1H, each s, *meso*-H). MS *m/z*: 681.2 $(M+H)^+$; IR (KBr) *v*: 3469 (N–H), 2964, 22929, 2856 (C–H), 1747–1710 (C=O), 1633 (C=C), 1556 (chlorin skeleton), 1465, 1396, 1263, 1003, 910, 806 cm^{-1}. Anal. calcd for $C_{41}H_{40}N_6O_4$: C 72.23, H 5.92, N 12.34; found C 72.29, H 5.70, N 12.55.

20-Chloro-*N*-methoxylpurpurin-18 imide methyl ester (5)

To a dichloromethane solution (15 mL) of **3b** (65 mg, 0.107 mmol), 16 mg *N*-chlorosuccinimide (0.120 mmol) was added and stirred under nitrogen. The reaction was monitored by TLC and stopped by a complete disappearance of starting material with a concomitant appearance of new spot. The mixture was poured into water, extracted with dichloromethane (2 × 20 mL) and evaporated to dryness under the vacuum. The residue was purified on chromatography on a silica gel column with hexane–ethyl acetate (4:1) to give 49 mg **5** (0.076 mmol, 71 %) as a red solid. 189–192 °C. UV–vis ($CHCl_3$) λ_{max}: 370 (relative intensity, 0.54), 424 (1.00), 576 (0.24), 618 (0.05), 722

(0.52) nm; ^{1}H NMR ($CDCl_3$) δ: −0.57 (1H, br s, NH), 0.32 (1H, br s, NH), 1.51 (3H, d, $J = 7.0$ Hz, 18-CH_3), 1.65 (3H, t, $J = 7.6$ Hz, 8a-CH_3), 1.96–2.08, 2.32–2.39, 2.53–2.61, 2.79–2.88 (each m, all 4H, 17a + 17b-H), 3.14, 3.58, 3.80, 4.34 (each s, each 3H, CH_3 + OCH_3), 3.61 (2H, t, $J = 7.6$ Hz, 8b-H), 4.78 (1H, q, $J = 7.0$ Hz, 18-H), 5.26 (1H, dd, $J = 9.1$, 2.6 Hz, 17-H), 6.11 (1H, d, $J = 17.8$ Hz, *trans*-3b-H), 6.24 (1H, d, $J = 11.5$ Hz, *cis*-3b-H), 7.80 (1H, dd, $J = 17.8$, 11.5 Hz, 3a-H), 9.53, 9.45 (each s, 2H, *meso*-H), MS *m/z*: 642.2 $(M + H)^+$; IR (KBr) *v*: 3460 (N–H), 2974, 2237 (C–H), 1741–1691 (C=O), 1685 (C=C), 1591 (chlorin skeleton), 1456, 1348, 1176, 1116, 1016,781 cm^{-1}. Anal. calcd for $C_{35}H_{36}ClN_5O_5$: C 65.46, H 5.65, N 10.91; found C 65.28, H 5.41, N 10.79.

3-(1,2-Dibromoethyl)-3-devinyl-*N*-methoxylpurpurin-18 imide methyl ester (6)

To a dichloromethane solution (50 mL) of **3b** (212 mg, 0.349 mmol), 65 mg *N*-bromosuccinimide (0.364 mmol) was added and stirred under nitrogen. The reaction was monitored by TLC and stopped when starting material disappeared completely. The mixture was poured into water, extracted with dichloromethane (2 × 20 mL) and evaporated to dryness under vacuum. The residue was purified on chromatography on a silica gel column with hexane–ethyl acetate (4:1) to give 142 mg **6** (0.185 mmol, 53 %) as a red solid: UV–vis m.p. 232–235 °C; UV–vis ($CHCl_3$) λ_{max}: 367 (relative intensity, 0.38), 417 (1.00), 508 (0.05), 547 (0.17), 620 (0.04), 707 (0.92) nm; ^{1}H NMR ($CDCl_3$) δ: -0.52 (-0.64) (1H, each br s, NH), −0.30 (−0.39) (1H, each br s, NH), 1.61 (1.62) (3H, t, $J = 7.6$ Hz, 8-CH_3), 1.68 (1.63) (3H, d, $J = 7.4$ Hz, 18-CH_3), 3.08–3.16, 2.25–2.38, 2.42–2.53, 2.73–2.80 (all 4H, each m, 17a + 17b-H), 3.16 (3.18), 3.36 (3.35), 3.60, 3.63 (3.61), 4.34 (4.33) (each 3H, each s, CH_3 + OCH_3), 3.57 (2H, q, $J = 7.6$ Hz, 8a-H), 4.07 (4.06) (1H, q, $J = 7.4$ Hz, 18-H), 4.32 (4.19) (1H, t, $J = 4.2$ Hz, 3b-H), 4.33 (4.23) (1H, t, $J = 9.2$ Hz, 3b-H), 5.12 (4.99) (1H, d, $J = 8.6$ Hz, 17-H), 6.31 (1H, dt, $J = 9.2$, 4.4 Hz, 3a-H), 8.47 (8.42), 9.25 (9.27), 9.70 (9.69) (each s, each 1H, meso-H), IR (KBr) *v*: 3471 (N–H), 2972, 2233 (C–H), 1741–1703 (C=O), 1623 (C=C), 1541 (chlorin skeleton), 1473, 1384, 1222, 1172, 1118, 912 cm^{-1}. Anal. calcd for $C_{35}H_{37}Br_2N_5O_5$: C 54.77, H 4.86, N 9.12; found C 54.60, H 5.04, N 9.30.

7-Formylpurpurin-18 methyl ester (7)

This compound was obtained in yield of 28 % according to the method for preparing compound **2**. m.p. 215–217 °C; UV–vis (CHCl3) λ_{max}: 412 (relative intensity, 1.00), 546 (0.14), 634 (0.06), 672 (0.23), 700 (0.19) nm; ^{1}H NMR (CDCl3) δ: −0.14 (1H, br s, NH), 0.36 (1H, br s, NH), 1.74 (3H, d, $J = 7.3$ Hz, 18-CH_3), 1.81 (3H, t, $J = 7.6$ Hz, 8-CH_3), 1.91–2.01, 2.44–2.54, 2.69–2.80 (all 4H, each m, 17a + 17b-H), 3.30, 3.61, 3.79 (each 3H, each s, OCH_3 + CH_3), 4.02 (2H, q, $J = 7.6$ Hz, 8a-H), 4.34 (1H, q, $J = 7.1$ Hz, 18-H), 5.14 (1H, d, $J = 7.8$ Hz, 17-H), 6.24 (1H, d, $J = 11.5$ Hz, *cis*-3b-H), 6.38 (1H, dd, $J = 17.8$ Hz, *trans*-3b-H), 7.87 (1H, dd, $J = 17.8$, 11.5 Hz, 3a-H), 8.48, 9.68, 10.32 (each 1H, each s, *meso*-H), 11.06 (s, 1H, CHO); MS *m/z*: 593.4 $(M + H)^+$; IR (KBr) *v*: 3460 (N–H), 2972, 2939 (C–H), 1741–1697 (C=O), 1662 (C=C), 1583 (chlorin skeleton), 1469, 1384, 1184, 1132, 993 cm^{-1}. Anal. calcd for $C_{34}H_{32}N_4O_6$: C 68.91, H 5.44, N 9.45; found C 68.77, H 5.61, N 9.58.

7-Styryl-*N*-methoxylpurpurin-18 imide methyl ester (8)

Chlorin **7** (124 mg, 0.209 mmol) and benzyltriphenylphosphonium chloride (100 mg, 0.257 mmol) was dissolved in 50 mL CH_2Cl_2 and a solution of NaOH (40 mg) in H_2O (15 mL) was added with stirring. The solution was stirred at room temperature under nitrogen for 1 h and poured into ice water and CH_2Cl_2. The aqueous phase was extracted with several portions of CH_2Cl_2 and the combined organic phases were acidized with aq. 2 % HCl, washed with aq. 4 % $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated *in vacuum* to dryness. The residue was redissolved in pyridine (10 mL), to which hydroxylamine hydrochloride (100 mg) was added. The resulting solution was stirred at room temperature for 12 h. The reaction mixture was dissolved in 20 mL of acetic anhydride, stirred for 4 h, poured into water and extracted with dichloromethane (3 × 100 mL). The combined extract was washed with saturated $NaHCO_3$ and water, dried over Na_2SO_4, methylated with diazomethane and evaporated to dryness. The crude product was purified by chromatography on silica gel column with hexane–ethyl acetate (4:1) to give 9 mg **8** (0.013 mmol, 6 %) as a red solid. m.p. 212–15 °C; UV–vis ($CHCl_3$) λ_{max}: 370 (relative intensity, 0.47), 432 (1.00), 4.67 (0.05), 564 (0.17), 6.37 (0.08), 730 (0.56) nm; ^{1}H NMR (400 MHz. $CDCl_3$) δ: 0.53 (1H, br s, NH), 0.47 (1H, br s, NH), 1.55 (3H, t, $J = 7.6$ Hz, 8-CH_3), 1.73 (3H, d, $J = 7.3$ Hz, 18-CH_3), 1.90–2.01, 2.43–2.58, 2.80–2.87 (all 4H, each m, 17a + 17b-H), 3.02, 3.26, 3.60, 4.36 (each 3H, each s, CH_3 + OCH_3), 3.38 (2H, q, $J = 7.6$ Hz, 8a-H), 4.31 (1H, q, $J = 7.4$ Hz, 18-H), 5.25 (1H, dd, $J = 9.6$, 2.2 Hz, 17-H), 6.12 (1H, d, $J = 11.5$ Hz, *cis*-3b-H), 6.22 (1H, d, $J = 17.5$ Hz, *trans*-3b-H), 7.44 (1H, t, $J = 7.3$ Hz, Ph-H), 7.53 (2H, t, $J = 7.3$ Hz, Ph-H), 7.72 (1H, d, $J = 16.5$ Hz, 7b-H), 7.78 (1H, dd, $J = 17.8$, 11.5 Hz, 3a-H), 7.81 (2H, t, $J = 7.3$ Hz, Ph-H), 8.68 (1H, d, $J = 16.5$ Hz, 7a-H), 8.42, 9.32, 9.42 (each 1H, each s, *meso*-H), MS *m/z*: 696.3 $(M + H)^+$; IR (KBr) *v*: 3446

(N–H), 2925, 2856 (C–H), 1741–1710 (C=O), 1612 (C=C), 1533 (chlorin skeleton), 1463, 1307, 1132, 1076, 1000 cm^{-1}. Anal. calcd for $C_{42}H_{41}N_5O_5$: C 72.50, H 5.94, N 10.07; found C 72.71, H 5.81, N 9.94.

12-Formyl-*N*-methoxylpurpurin-18 imide methyl ester (9)

To a THF solution (25 mL) of **3b** (232 mg, 0.346 mmol), an aqueous solution (3 mL) of LiOH (600 mg) and methanol (8 mL) were sequentially added. This mixture was violently stirred in open system in dark for 3 h, poured into cool water, adjusted pH to 3 with sulfuric acid and then extracted with dichloromethane (2 × 50 mL). The combined extract was washed with water, dried over anhydrous Na_2SO_4 and treated with CH_2N_2 for short time (approximately 5 min). After evaporation in vacuo, the residue was purified on chromatography on a silica gel column with hexane–ethyl acetate (5:1) to give 147 mg **9** (0.118 mmol, 34 %) as a bluish-green solid. m.p. >300 °C; UV–vis ($CHCl_3$) λ_{max}: 379 (relative intensity, 0.70), 423 (1.00), 585 (0.27), 659 (0.24), 715 (0.44) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ: −0.06 (1H, br s, NH), 0.80 (1H, br s, NH), 1.60 (3H, t, $J = 7.6$ Hz, 8-CH_3), 1.67 (3H, d, $J = 7.3$ Hz, 18-CH_3), 1.85–1.95, 2.36–2.45, 2.48–2.58, 2.76–2.86 (all 4H, each m, 17a + 17b-H), 2.97, 3.22, 3.62, 4.35 (each 3H, each s, CH_3 + OCH_3), 3.49 (2H, q, $J = 7.6$ Hz, 8a-H), 4.18 (1H, q, $J = 7.4$ Hz, 17-H), 5.07 (1H, d, $J = 9.1$ Hz, 17-H), 6.16 (1H, d, $J = 11.6$ Hz, *cis*-3b-H), 6.23 (1H, d, $J = 17.8$ Hz, *trans*-3b-H), 7.68 (1H, dd, $J = 17.8$, 11.6 Hz, 3a-H), 8.16, 8.79, 10.21 (each 1H, s, *meso*-H), 11.78 (s, 1H, 12-CHO); mass m/z: 622.3 (M + 1); IR (KBr) *v*: 3506, 3332 (N–H), 2962, 2931 (C–H), 1737–1701 (C=O), 1662 (C=C), 1563 (chlorin skeleton), 1444, 1367, 1251, 1076, 908 cm^{-1}. Anal. calcd for $C_{35}H_{35}N_5O_6$: C 67.62, H 5.67, N 11.27; found C 67.68, H 5.47, N 11.13.

Nickel(II) 3b-formylpurpurin-18 imide methyl ester (11)

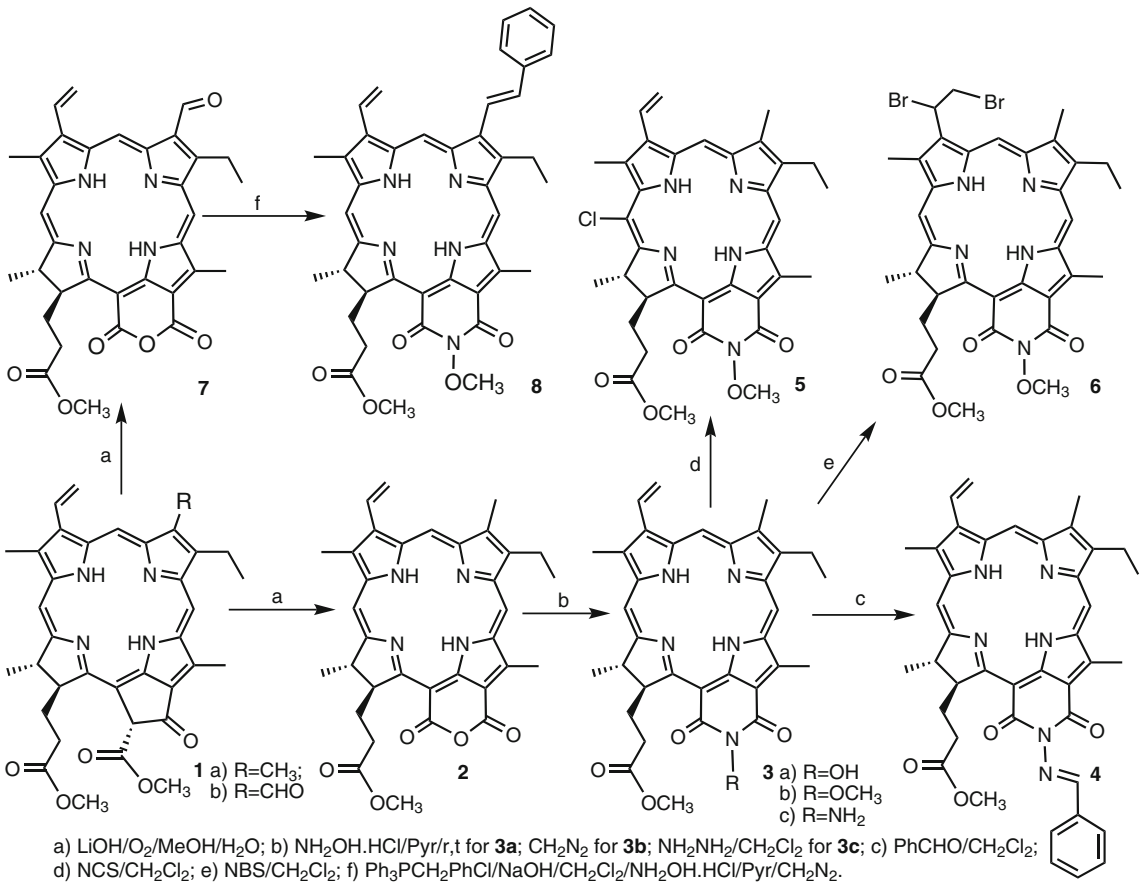
To a saturated solution of $Ni(AcO)_2$ in methanol (10 mL) 168 mg chlorin **3b** (0.277 mmol) was added in methylenechloride (20 mL). The mixture was then stirred at 50 °C for 12 h. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was chromatographed on silica gel with hexane–ethyl acetate (4:1) to give 140 mg nickel complex (0.211 mmol) **10** in 76 % yield, which was directly used for the next Vilsmeier reaction without further identification. Phosphorus oxychloride (52 mg, 0.315 mmol) was added dropwise to a solution of *N*,*N*-dimethylformamide (23 mg, 0.315 mmol), and the mixture was stirred at 0 °C for 15 min. This mixture was then added to a solution of above mentioned nickel chlorin in dichloromethane (20 mL) with continuous stirring at 0 °C. The resultant mixture was then warmed up to room temperature and stirred for 18 h, to which saturated aqueous sodium carbonate (100 mL) was added. After stirring overnight the reaction mixture was extracted with dichloromethane. The combined organic layers were washed with water (3 × 100 mL), dried over Na_2SO_4 and evaporated in vacuo. The residue was purified on chromatography on a silica gel column with hexane–ethyl acetate (5:1) to give 60 mg **11** (0.086 mmol, 31 %) as a bluish-green solid. m.p. >300 °C; UV–vis ($CHCl_3$) λ_{max}: 417 (relative intensity, 1.00), 478 (0.10), 508 (0.17), 545 (0.57), 703 (0.38) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.32 (3H, d, $J = 7.1$ Hz, 18−CH_3), 1.52 (3H, t, $J = 7.6$ Hz, 8−CH_3), 1.89–1.94, 2.18–2.26, 2.28–2.71, 2.74–2.83 (all 4H, each m, 17a + 17b-H), 2.97, 3.09, 3.50, 4.64, 4.26 (each 3H, each s, CH_3 + OCH_3), 3.43 (2H, qd, $J = 19.7$, 7.6 Hz, 8a-H), 4.11 (1H, q, $J = 7.1$ Hz, 17-H), 4.71 (1H, dd, $J = 9.6$, 2.5 Hz, 17-H), 6.97 (1H, d, $J = 16.1$, 7.5 Hz, 3b-H), 8.33 (1H, d, $J = 16.1$ Hz, 3a-H), 8.01,8.73, 9.02 (each 1H, s, *meso*-H), 9.97 (d, $J = 7.5$ Hz, 1H, 3c-CHO); IR (KBr) *v*: 3506 (N–H), 2974, 2939 (C–H), 1731–1697 (C=O), 1608 (C=C), 1548 (chlorin skeleton), 1409, 1257, 1068, 910 cm^{-1}; Anal. calcd for $C_{36}H_{35}N_4NiO_6$: C 62.45, H 5.10, N 10.11; found C 62.68, H 5.17, N 9.97.

Results and discussion

Methyl pheophorbide-*a* **1a** (MP*a*) was used as starting material and successively treated with saturated methanol solution of LiOH in the presence of oxygen (exposure of the reaction mixture to air), acidified with AcOH and methylated with CH_2N_2 to produce purpurin-18 methyl ester **2** in 70 % yield. The treatment of this chlorophyll-degraded product with hydroxylamine hydrochloride afforded purpurin-18 imide **3a** in 82 % yield, and ulterior methylation with diazomethane almost quantitatively give, *N*-methoxyl purpurin-18 imide **3b**. The aminolysis reaction of purpurin-18 **2** smoothly generated *N*-amino-substituted pyrpurin-18 imide **3c** in 62 %. In order to further increase the absorption maximum band (Qy) the different chromophore or auxochrome groups were introduced along the periphery of macrocyclic molecular. Firstly, the hydrazone-forming of **3c** with benzaldehyde was carried out by stirring in dichloromethane in the presence of *p*-toluene sulphonic acid as catalyst to generate *N*-benzyliminopurpurin-18 imide **4** in 58 % yield. And then, electrophilic substitution of the **3b** with *N*-chlorosuccinimide (NCS) was performed in CH_2Cl_2 at room temperature to form 20-chloropurpurin imide **5** in good yield. Upon being subjected to the bromination reaction by treating with *N*-bromosuccinimide

(NBS) in dichloromethane at room temperature at same 20-*meso*-position of **3b** produced C3-bromine-adducted product **6** in 53 % yield without 20-bromine-substituted chlorin. Compared to chlorophyll-*a*, there has been relatively little research on modifications for chlorophyll-*b* which was also obtained from *Spirulina maxima* alga in one-fourth as long as chlorophyll-*a*'s yield. In order to make use of the formyl group attached to 7-position of chlorophyll-*b*, for the synthesis of novel C7-substituted chlorins with long-wavelength absorption, methyl pheophorbide-*b* **1b** (MP*b*), chosen as a precursor in another synthetic route, was converted into 7-formyl-purpurin-18 **7** in relatively lower yield (28 %) by allomerization according to the procedure for preparing compound **2**. Considering the extension of conjugated π-system of chromophore can effectively improve molecular absorption wavelength the successive reaction of compound **7**, including Wittig reaction with benzyltriphenylphosphonium chloride in the presence of aqueous sodium hydroxide and amination reaction with hydroxylamine hydrochloride, were performed in one-pot method, and C7-*trans*-styryl substituted chlorin **8** was separated from quite mixed products in poor yield (Scheme 2).

The chlorin linked with special functional group frequently display combinatorial properties associated with the joint moiety. For example, *β*-galactose-conjugated chlorins exhibit excellent solubility in water (PDT) [24], and chloro-phyll-*a* analogues conjugated with aminobenzyl-Gd(III)-DTPA can be used as potential bifunctional agents for magnetic resonance imaging and photodynamic therapy [25]. Constructing highly active functional groups along periphery of chlorin is a important synthetic strategy to acquire ideal photosensitizer used in PDT, therefore we attempted introducing formyl group at C12-position and formylvinyl at 20-*meso*-position. 12-Formylpurpurin-18 imide **9** was obtained in 34 % yield from **3b** by successive treatment with saturated methanol solution of LiOH in the presence of oxygen (exposure of the reaction mixture to air), acidification with AcOH and methylation with CH_2N_2. Upon treatment with excess nickel acetate in MeOH by refluxing, chlorin **3b** was converted into its nickel complex **10** which was directly used for the next reaction without further separation and purification. The formylation of this nickel(II) complex was performed by the Vilsmeier reaction with *N*, *N*-dimethylformamide (DMF) in the presence of phosphorus oxychloride and basic hydrolysis and



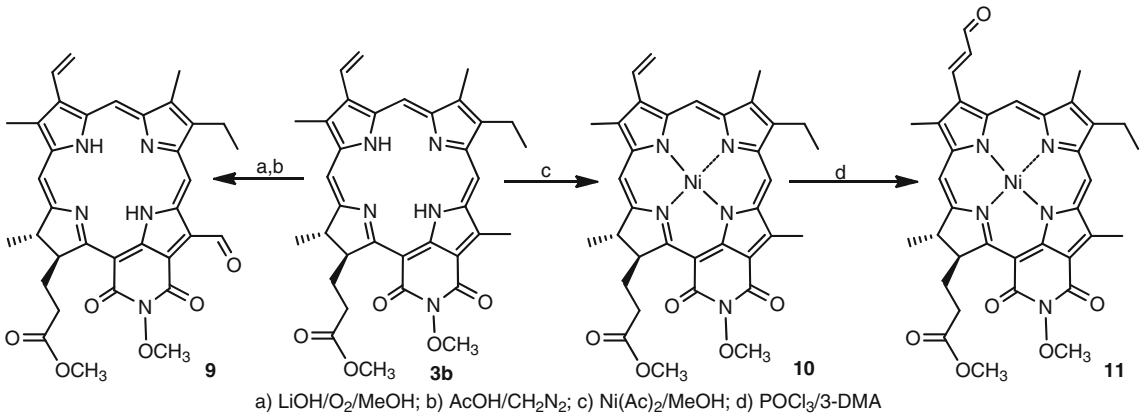
Scheme 2 Synthetic routes of chlorophyll a and b derivatives

neutralization to afford nickel(II) 3b- formyl-substituted purpurin-18 imide **11** as a major product in 31 % yield (Scheme 3).

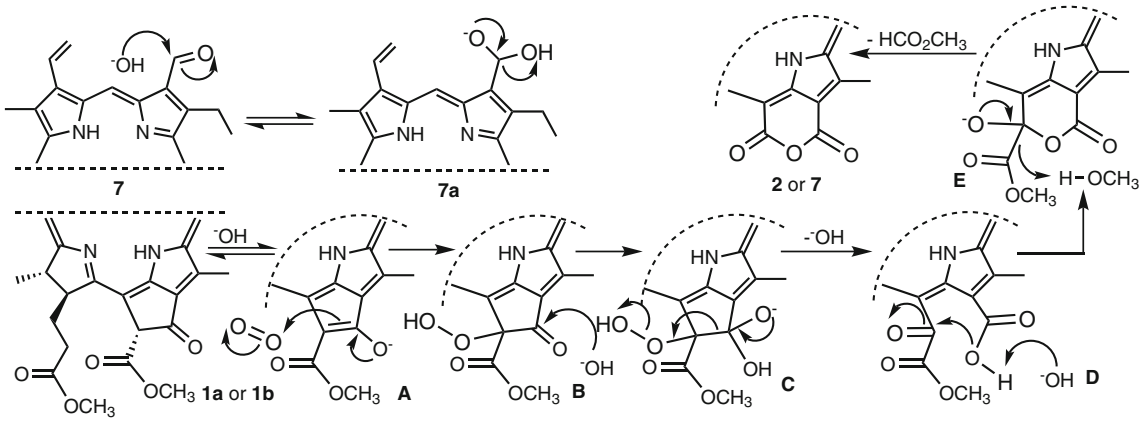
The ring-opening of exocyclic ring is a typical reaction of methyl pheophorbide-*a* due to its rigid ring-structure and *β*-keto ester functional group [26–30]. In alkaline condition, MP*a* **1a** can be isomerized into its enol form isomer **A,** which reacted with the oxygen molecule in the air to give intermediate **B** linked with a peroxide band at 13^2-position. The carbonyl group in *E*-ring of this oxidization product was converted into tetrahedral intermediate **C** by nucleophilic attack of hydroxyl ion. Purpurin-7 diester **D**, formed from **C** by ring-opening reaction and leaving a hydroxyl ion, readily closed its exocyclic six-membered-ring by the intramolecular nucleophilic addition of C13-carboxyl group with C15a-carbonyl group to produce intermediate **E**. Finally, purpurin-18 **2** or 7-formyl- purpurin-18 **7** was generated by the re-arrangement reaction of **E** accompanied by leaving a methyl formate. In same condition, the formyl group attached to the 7-position of compound **7** was not oxidized by air and only just maintained at the equilibrium with **7a** (Scheme 4).

The electrophilic substitution of chlorophyll derivatives possessed excellent selectivities. Almost all aromatic electrophilic substitution on the chlorin chromophore occurred at 20-*meso*-position whose charge density was relatively higher because of linking with dihydropyrrole ring (ring D) [31, 32]. The chlorine atom was introduced into 20-position in the chlorination of compound **3b** as previous reported electrophilic substitutions [26–33].

The variation of exocyclic ring from pheophorbide to purpurin-18 and its imide adequately extends the π-system of macrocyclic molecule. All the purpurin-18 imide displayed their Qy peak above 700 nm. It was found that the moieties attached to the nitrogen atom of imide could also influence on molecular UV–vis spectra. The reason for longer Qy-wavelength possessed by compound **3c**, comparing with ones of other purpurin-18 mides such as **3b** or **4**, was that amino group connected to nitrogen atom could form a hydrogen bond with adjacent carbonyl group to

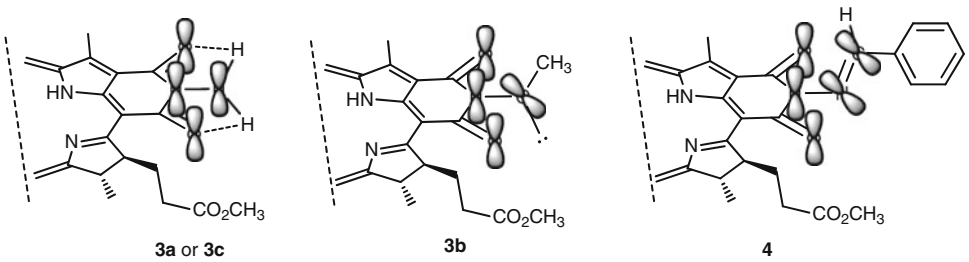


Scheme 3 Synthetic routes of purpurinimide and its metal complex



Scheme 4 Possible mechanism of air-oxidation of MPa and MPb under basic condition

Scheme 5 Possible mechanism of the Qy spectra changes of the purpurinimides



keep its isolated electron pair paralleling with the *p*-orbital of the exocyclic ring as possible. On the contrary, the other imide was not capable of causing such hydrogen bond with 13^1- or 13^3-carbonyl group and their *p*-orbitals of the heteroatom, joined to the nitrogen atom of imide ring, skewed off the macrocyclic plane due to the repulsion between the *N*-substituted groups and the carbonyl group in the exocyclic ring. About the same as chlorin **3c**, the Qy peak of **3a** was 12 nm in length than the ones of **3b** because the hydroxyl group on the exocyclic ring, could form hydrogen-bond with adjacent carbonyl groups (Scheme 5).

The aza-annulene aromatic structure of purpurin-18 imide is capable of forming a ring current flowed along its π-system which causes intense shield and deshield effect to the functional group linked with inner ring and outer ring, respectively. The signals of methyl groups linked with chlorin periphery basically all appeared beyond, 3.00 ppm which indicated the exocyclic substituted moieties received a strong deshield effect coming from ring current. Contrary to it, the protons bonded to central nitrogen atoms were strong shielded and moved to higher-field [34]. The enlargement of exocyclic ring from five-membered cycloketone to six-membered imide brought the carbonyl group at 15-position cause intense deshield effect on adjacent single C17-proton. The ^{1}H NMR spectrum of all purpurin-18 derivatives clearly showed the signals of C17-protons appeared above 5.00 ppm.

In conclusion, the synthetic methodology constructing purpurin-18 imide of chlorin possessing chlorophyll skeleton from pheophorbide and performing chemical modification along the periphery of chromophore lay the groundwork for finding new photosensitizer with long-wavelength absorption. A series of common reactions, such as electrophilic substitution, Wittig reaction, allomerization and Vilsmeier reaction, introduced conveniently a variety of substituted groups which were capable of conjugating with macrocycle. These modifications for increasing molecular absorption maximum (Qy) may be valuable in developing new generation of photosensitizers for PDT.

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References

1. J.J. Wang, Chin. J. Org. Chem. **25**, 1353 (2005)
2. V.Y. Pavlov, G.V. Ponomarev, Chem. Heterocyclic Compt. **40**(4), 393 (2004)
3. A.N. Kozyrev, Y.H. Chen, L.N. Goswami, W.A. Tabaczynski, R.K. Pandey, J. Org. Chem. **71**, 1949 (2006)
4. A.F. Mironov, M.A. Grin, D.V. Dzardanov, K.V. Golovina, Y.K. Shim, Mendeleev. Commun. **11**, 205 (2001)
5. A.P. Castano, T.N. Demidova, M.R. Hamblin, Photodiagn. Photodyn. Ther. **1**, 279 (2004)
6. A.P. Castano, T.N. Demidova, M.R. Hamblin, Photodiagn. Photodyn. Ther. **2**, 91 (2005)
7. G. Zheng, W.R. Potter, S.H. Camacho, J.R. Missert, G.S. Wang, D.A. Bellnier, B.W. Henderson, M.A.J. Rodgers, T.J. Dougherty, R.K. Pandey, J. Med. Chem. **44**, 1549 (2001)
8. Y.H. Chen, G.L. Li, R.K. Pankey, Curr. Org. Chem. **8**, 1105 (2004)
9. A.N. Kozyrev, J.L. Alderfer, T. Srikrishnan, R.K. Pandey, J. Chem. Soc. Perkin Trans. **1**, 837 (1998)
10. A.N. Kozyrev, V. Suresh, M.O. Senge, M. Shibata, T.J. Doughertya, R.K. Pandeya, Tetrahedron. **56**, 3353 (2000)
11. A. N. Kozyrev, J. L. Alderfer, T. J. Doughertya, R. K. Pandey, Chem. Commun. **4**, 1083 (1998)
12. J. J. Wang, G. F. Han, Y. K. Shim, J. Iran. Chem. Soc. **8**, 965 (2011)
13. H. Tamiaki, T. Miyatake, R. Tanikaga, Tetrahedron Lett. **38**, 267 (1997)
14. G.F. Han, J.J. Wang, X.J. Chang, Chin. J. Org. Chem. **24**, 197 (2004)
15. K.M. Smith, D.A. Goff, D.G. Simposon, J. Am. Chem. Soc. **107**, 4946 (1985)
16. J.Z. Li, W.H. Liu, F.G. Li, J.J. Wang, Y.R. Sou, Y.J. Liu, Chin. J. Org. Chem. **27**, 1594 (2007)
17. J. J. Wang, J. Z. Li, F. G. Li J, Iran. Chem. Soc. **8**, 1139 (2011)
18. J.J. Wang, Y.K. Shim, G.J. Jiang, K. Imafuku, J. Heterocycl. Chem. **41**, 29 (2004)
19. J.J. Wang, Y.K. Shim, J.G. Jiang, K. Imafuku, J. Heterocycl. Chem. **40**, 1075 (2003)
20. J. J Wang, P. Wang, J. Z. Li, J. Jakus, Y. K. Shim, Bull. Korean Chem. Soc. **32**, 3473 (2011)
21. J. Wu, J.G. Yin, Q. Zhang, C.M. Sun, F.G. Li, W. Pei, J.J. Wang, Chin. J. Org. Chem. **31**, 2011 (2011)
22. S.H. Lee, N. Jauervic, K.M. Smith, J. Chem. Soc. Perkin Trans. **1**, 837 (1993)
23. G.F. Han, J.J. Wang, Y. Qu, Y.K. Shim, Chin. J. Org. Chem. **26**, 43 (2006)
24. G. Zheng, A. Graham, M. Hibata, J.R. Missert, A.R. Oseroff, T.J. Dougherty, R.K. Pandey, J. Org. Chem. **66**, 8709 (2001)
25. G. Li, A. Slansky, M.P. Dobhal, L.N. Goswami, A. Graham, Y.H. Chen, P. Kanter, R.A. Alberico, J. Spernyak, J. Morgan, R. Mazurchuk, A. Oseroff, Z. Grossman, R.K. Pandey, Bioconjugate Chem. **16**, 32 (2005)

26. X.R. Wu, C. Liu, Z. Yang, N.N. Yao, J.J. Wang, Chin. J. Org. Chem. **32**, 632 (2012)
27. J.G. Yin, Z. Wang, Z. Yang, C. Liu, L.L. Zhao, J.J. Wang, Chin. J. Org. Chem. **32**, 360 (2012)
28. R.G.W. Jinadasa, X.K. Hu, M.G. Vicente, K.M. Smith, J. Med. Chem. **54**, 7464 (2011)
29. J.Z. Li, J.J. Wang, I. Yoon, B.C. Cui, Y.K. Shim, Bioorg. Med. Chem. Lett. **22**, 1846 (2012)
30. J.J. Wang, J.Z. Li, J. Jakus, Y.K. Shim, J. Porphyrins Phthalocyanines **16**, 123 (2012)
31. J.J. Wang, C.L. Liu, J.Z. Li, Synth. Commun. **42**, 487 (2012)
32. J.J. Wang, C.L. Liu, J.Z. Li, Synth. Commun. **43**, 487 (2012)
33. H. Tamiaki, Y. Kotegawa, K. Mizutani, Bioorg. Med. Chem. Lett. **18**, 6037 (2008)
34. R.J. Abraham, K.M. Shimth, D.A. Goff, J.J. Lai, J. Am. Chem. Soc. **104**, 4332 (1982)