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Synthesis and properties of the Zn-chlorin–bacteriochlorin dimer

Mikhail A. Grin,*[a] Ivan S. Lonin,[a] Sergey V. Fedyunin,[a] Alexander G. Tsiprovskiy,[a] Anton A. Strizhakov,[a,b] Anatoly A. Tsygankov,[c] Alexander A. Krasnovsky[b] and Andrey F. Mironov[a]

[a] *M. V. Lomonosov Moscow State Academy of Fine Chemical Technology, 119571 Moscow, Russian Federation. Fax: +7 495 936 8901; e-mail: michael_grin@mail.ru*
[b] *A. N. Bach Institute of Biochemistry, Russian Academy of Sciences, 119071 Moscow, Russian Federation*
[c] *Institute of Basic Biological Problems, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russian Federation*

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A chlorin–bacteriochlorin dimer containing a Zn^{II}-complex of a chlorin e_6 derivative covalently bound with the cycloimide of bacteriochlorin *p via* a spacer group was obtained.

The ability of porphyrins to show selective accumulation in tumors and the subsequent intense fluorescence under irradiation with generation of singlet oxygen opens up real prospects for the fluorescent diagnostics (FD)[1–3] and photodynamic therapy (PDT) of tumors.[4]

Derivatives of bacteriochlorophyll *a* having strong absorption in the near-IR region 770–840 nm have proved well as photodynamic agents. Owing to the enhanced 'transparency' of tissues in this range, this makes it possible to affect tumors located at depths of up to 15–20 mm (compared to 2–3 mm for porphyrins). Furthermore, bacteriochlorin derivatives have low dark toxicity; they are well accumulated in tumors and quickly excreted from the body.[5–13] On the other hand, it is known that metal complexes of porphyrins and chlorins find use as fluorescent labels but have low efficiency in PDT.[14]

This study was aimed at the synthesis of a dimer consisting of chlorin and bacteriochlorin bound *via* a spacer to ensure their joint accumulation in a tumor.

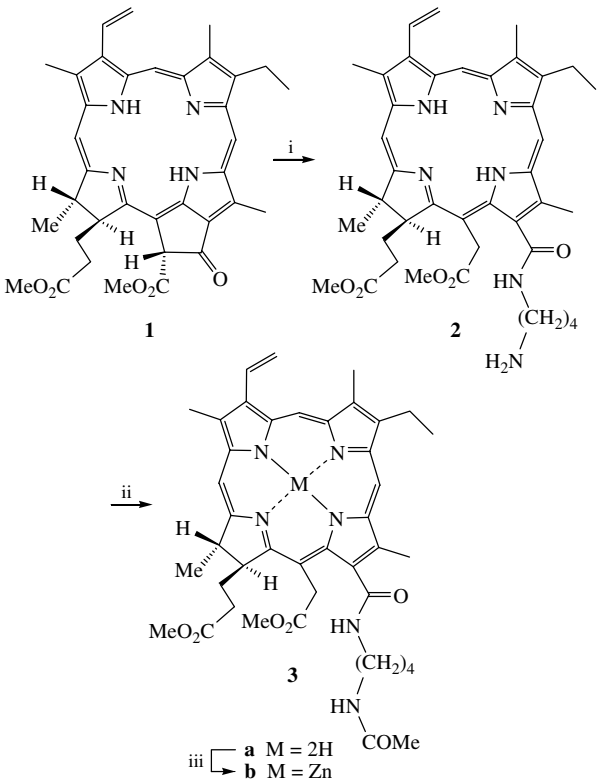
We used the Zn^{II}-complex of chlorin e_6 as the chlorin component. Published data are available on the incorporation of a zinc atom in chlorins and bacteriochlorins, and it was noted that chlorins require milder complexation conditions.[15]

The spacer was incorporated in the pheophorbide *a*[16] by opening the pentanone exocycle in pheophorbide **1** on treatment with 1,4-diaminobutane (Scheme 1).† The presence of a spacer group between the dimer subunits is necessary to minimise spatial interactions between the macrocycles that could quench fluorescence and decrease the photocytotoxicity of the sensitizer. The progress of the reaction was monitored by TLC: resulting compound **2** had a much lower mobility than the original pheophorbide, while the electronic spectrum showed an insignificant hypsochromic shift of the Q band from 668 to 662 nm.

The structure of the product was confirmed by its mass spectrum, whereas the ^{1}H NMR spectrum was noninformative, presumably, due to the low solubility of the compound and its trend to form aggregates. We solved this problem by converting the free amine into its acyl analogue by treatment of chlorin **2** with acetic anhydride. Reaction product **3a** had a much higher chromatographic mobility than the original amide and underwent chromatography more readily. The structure of the resulting acetylated analogue was confirmed by mass and ^{1}H NMR spectra. Chlorin **3a** was converted to Zn^{II}-complex **3b**.

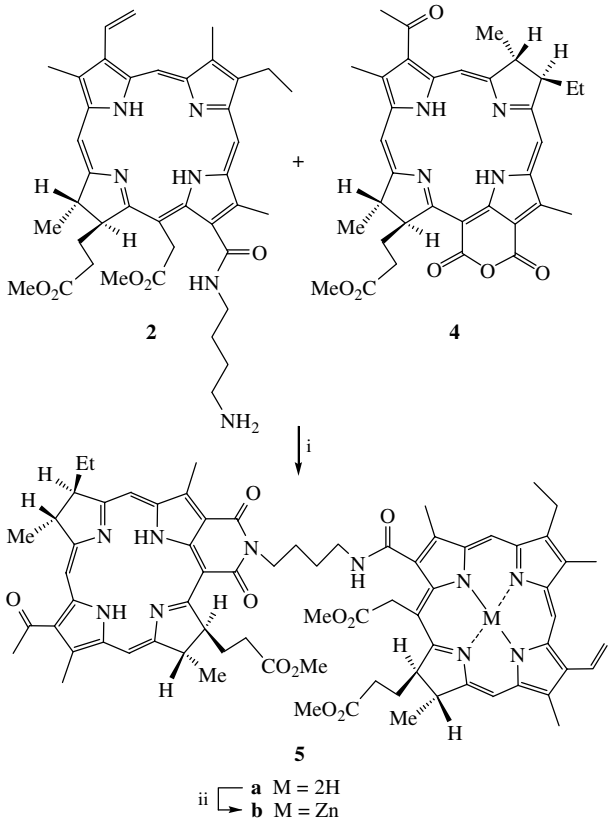
Complexation was monitored spectrophotometrically using the hypsochromic shift of the absorption maximum from 662 to 636 nm (Figure 1). Irradiation of Soret band resulted in the intense fluorescence of Zn-chlorin **3b** at 646 nm with a quantum yield of about 40% (Figure 2).

Synthesis of the target dimer (Scheme 2) included the reaction of chlorin **2** with the bacteriopurpurin methyl ester **4**, which involved opening of the anhydride cycle and formation of isomeric amides.[17] Treatment of the reaction mixture with diazomethane gave the corresponding methyl esters having high chromatographic mobility, which made their purification more efficient. The electronic spectrum of the dimers contains absorption bands characteristic of chlorin (660 nm) and bacteriochlorin subunits (762 nm).



Scheme 1 *Reagents and conditions*: i, $NH_2(CH_2)_4NH_2$, 20 °C, 5 h; ii, Ac_2O; iii, $(MeCOO)_2Zn$, MeOH, 20 °C, 30 min.

Subsequent intramolecular cyclization on treatment with methanolic alkali[17] gave dimer **5a**. A bathochromic shift of the long-wave absorption maximum from 762 to 821 nm was observed during the reaction (Figure 1). The mass spectrum of dimer **5a** confirmed the formation of an imide ring, judging by the decrease in mass by one methanol molecule in comparison with the original compounds.



Scheme 2 *Reagents and conditions*: i, $CHCl_3$, reflux, 4 h; CH_2N_2, Et_2O, 20 °C, 15 min; KOH–MeOH, 20 °C, 10 min; ii, $(MeCOO)_2Zn$, MeOH, 20 °C, 30 min.

The ^{1}H NMR spectrum of dimer **5a** is very complex, so we had to use two-dimensional NMR spectroscopy methods, COSY and HSQC, to assign the signals of all protons. Since the complexation rate is considerably higher for chlorins than for bacteriochlorins, we were able to selectively incorporate a metal ion into the dimer chlorin component (Scheme 2). Dimer **5a** was treated with a solution of zinc acetate in methanol at room temperature. The formation of the Zn^{II}-complex of chlorin was confirmed by electronic, mass and ^{1}H NMR spectra.

The mass spectrum contains a molecular ion signal with a characteristic set of signals of zinc isotopes. The ^{1}H NMR spectrum of dimer **5b** contains no signals of NH-protons of the chlorin macrocycle. The electronic absorption spectrum (Figure 1) shows a hypsochromic shift of the absorption band from 664 to 636 nm and a bathochromic shift of the Soret band from 403 to 414 nm, which is explained by the effect of the central zinc ion on the electron density distribution in

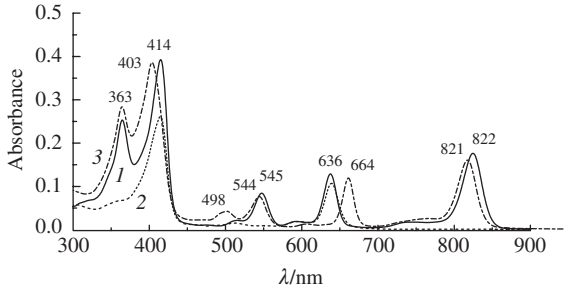


Figure 1 Electronic absorption spectra in benzene: (*1*) zinc complex **5b**; (*2*) zinc complex **3b**; (*3*) **5a**.

† Pheophorbide *a* and bacteriopurpurin were obtained using conventional techniques.[19,20]

2: yield 70%. MS (MALDI), *m*/*z*: 695 (M^+). UV-VIS [$CHCl_3$, λ/nm (relative intensities)]: 402 (Soret), 526, 609 and 662 (1:0.03:0.04:0.30).

3a: yield 92%. MS (ESI), *m*/*z*: 737 (M^+). UV-VIS [$CHCl_3$, λ/nm (relative intensities)]: 402 (Soret), 526, 609 and 662 (1:0.03:0.04:0.30). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.72 (s, H, 5-H), 9.67 (s, H, 10-H), 8.90 (s, H, 20-H), 8.03 (dd, H, 3^1-H, *J* 18 and 12 Hz), 7.08 (H, 13^2-NH), 6.31 (d, H, *E*-3^2-H, *J* 18 Hz), 6.19 (H, 13^7-NH), 6.31 (d, H, *Z*-3^2-H, *J* 12 Hz), 5.55 and 5.20 (d, 2H, 15-CH_2), 4.50 (q, H, 18-H), 4.39 (d, H, 17-H, *J* 9 Hz), 3.76 (m, 2H, 8^1-CH_2), 3.75 (s, 3H, 15^2-CO_2Me), 3.62 (s, 3H, 12-Me), 3.50 (s, 3H, 17^3-CO_2Me), 3.48 (s, 5H, 2-Me) (m, 2H, 13^3-CH_2), 3.29 (s, 3H, 7-Me), 3.19 (m, 2H, 13^6-CH_2), 2.56 (m, H, 17^1-CH_2), 2.20 (m, 3H, 17^1-CH_2, 17^2-CH_2), 1.85 (s, 3H, COMe), 1.74 (d, 3H, 18-Me, *J* 7 Hz), 1.67 (t, 3H, 8^2-Me, *J* 8 Hz), 1.12 (m, 4H, 13^4-CH_2, 13^5-CH_2), –1.61 (H, NH), –1.84 (H, NH).

3b: yield 95%. MS (ESI), *m*/*z*: 737 (M^+ – Zn), 799 (M+). UV-VIS [$CHCl_3$, λ/nm (relative intensities)]: 407 (Soret), 507, 566 and 636 (1:0.05:0.06:0.30). ^{1}H NMR (300 MHz, CD_3OD) δ: 9.59 (s, H, 5-H), 9.51 (s, H, 10-H), 8.62 (s, H, 20-H), 8.09 (dd, H, 3^1-H, *J* 18 and 12 Hz), 6.18 (d, H, *E*-3^2-H, *J* 18 Hz), 5.95 (H, 13^7-NH), 6.31 (d, H, *Z*-3^2-H, *J* 12 Hz), 5.53 and 5.18 (d, 2H, 15-CH_2), 4.46 (q, H, 18-H), 4.29 (d, H, 17-H, *J* 9 Hz), 3.82 (m, 2H, 8^1-CH_2), 3.75 (s, 3H, 15^2-CO_2Me), 3.64 (s, 3H, 12-Me), 3.52 (m, 2H, 13^3-CH_2), 3.38 (s, 3H, 17^3-CO_2Me), 3.36 (s, 3H, 2-Me), 3.29 (s, 3H, 7-Me), 3.22 (m, 2H, 13^6-CH_2), 2.67 (m, H, 17^1-CH_2), 2.25 (m, 3H, 17^1-CH_2, 17^2-CH_2), 1.95 (s, 3H, COMe), 1.78, (m, 4H, 13^4-CH_2, 13^5-CH_2), 1.71 (t, 3H, 8^2-Me, *J* 8 Hz), 1.61 (d, 3H, 18-Me, *J* 7 Hz).

5a: yield 30%. UV-VIS [benzene, λ/nm (relative intensities)]: 363, 403 (Soret), 498, 544, 664 and 821 (0.73:1:0.10:0.19:0.30:0.41). MS (MALDI), *m*/*z*: 1273.6 (M^+). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.75 (2H, 5_{Chl}-H, 10_{Chl}-H), 9.20 (s, H, 5_{Bchl}-H), 8.91 (s, H, 20_{Chl}-H), 8.75 (s, H, 10_{Bchl}-H), 8.54 (s, H, 20_{Bchl}-H), 8.09 (dd, H, 3^1-H, *J* 18 and 12 Hz), 6.36 (d, H, *E*-3^2-H, *J* 18 Hz), 6.18 (d, H, *Z*-3^2-H, *J* 12 Hz), 5.67 and 5.28 (d, 2H, 15-CH_2), 5.21 (dd, H, 17_{Bchl}-H, *J* 8 and 3 Hz), 4.61 (t, 2H, N-CH_2, *J* 7 Hz), 4.49 (m, H, 18_{Chl}-H), 4.40 (m, H, 17_{Chl}-H), 4.28 (qd, H, 7_{Bchl}-H, *J* 7.5 and 3 Hz), 4.12 (q, H, 18_{Bchl}-H, *J* 7.6 Hz), 4.07 (m, 3H, 8_{Bchl}-H, HN–CH_2), 3.83 (s, 3H, 12_{Bchl}-Me), 3.79 (m, 2H, 8_{Chl}-CH_2), 3.62 (s, 3H, 17^5_{Bchl}-Me), 3.59 (s, 3H, 2_{Bchl}-Me), 3.58 (s, 3H, 15^4_{Bchl}-Me), 3.50 (s, 3H, 17^5_{Chl}-Me), 3.49 (s, 3H, 2_{Chl}-Me), 3.45 (s, 3H, 12_{Chl}-Me), 3.15 (s, 3H, 3^2_{Bchl}-Me), 3.07 (s, 3H, 7_{Chl}-Me), 2.50 (m, 2H, 1H from 17^2_{Bchl}-CH_2 and 1H from 17^5_{Chl}-CH_2), 2.33 (m, 2H, 1H from 17^2_{Bchl}-CH_2 and 1H from 8_{Bchl}-CH_2), 2.28 (m, 3H, 1H from 17^1_{Bchl}-CH_2 and 2H from NCH_2CH_2), 2.15 (m, 5H, 4H from $NCH_2CH_2CH_2CH_2$ and 1H from 17^2_{Chl}-CH_2), 2.04 (m, 1H, 8_{Bchl}-CH_2), 1.85 (m, 1H, 17^1_{Bchl}-CH_2), 1.79 (d, 3H, 7_{Bchl}-Me, *J* 7.6 Hz), 1.74 (br., 5H, 3H from 18_{Chl}-Me and 2H from 17^1_{Chl}), 1.67 (t, 3H, 8_{Chl}-Me, *J* 7.6 Hz), 1.61 (d, 3H, 18_{Bchl}-Me, *J* 7.2 Hz), 1.09 (t, 3H, 8_{Bchl}-Me, *J* 8 Hz), –0.51 (H, N_{BchlH}), –0.74 (H, N_{BchlH}), –1.61 (H, N_{ChlH}), –1.84 (H, N_{ChlH}).

5b: yield 70%. UV-VIS [benzene, λ/nm (relative intensities)]: 363, 414 (Soret), 545, 636 and 822 (0.64:1:0.21:0.31:0.44). MS (MALDI), *m*/*z*: 1334 (M^+), 1273 (M^+ – Zn). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.55 (2H, 5_{Chl}-H, 10_{Chl}-H), 9.18 (s, 1H, 5_{Bchl}-H), 9.01 (s, 1H, 20_{Chl}-H), 8.74 (s, 1H, 10_{Bchl}-H), 8.50 (s, 1H, 20_{Bchl}-H), 8.08 (dd, 1H, 3^1-H, *J* 18 and 12 Hz), 6.37 (d, 1H, *E*-3^2-H, *J* 18 Hz), 6.21 (d, 1H, *Z*-3^2-H, *J* 12 Hz), 5.66 and 5.26 (d, 2H, 15-CH_2), 5.23 (m, 1H, 17_{Bchl}-H), 5.13 (m, 2H, N-CH_2), 4.57 (m, 2H, 18_{Chl}-H, 17_{Chl}-H), 4.27 (m, 1H, 7_{Bchl}-H), 4.07 (m, 1H, 18_{Bchl}-H), 3.99 (m, 3H, 8_{Bchl}-H, HN–CH_2), 3.82 (s, 3H, 12_{Bchl}-Me), 3.77 (m, 2H, 8_{Chl}-CH_2), 3.63 (s, 3H, 17^5_{Bchl}-Me), 3.55 (s, 6H, 2_{Bchl}-Me, 15^4_{Bchl}-Me), 3.49 (s, 3H, 17^5_{Chl}-Me), 3.45 (s, 3H, 12_{Chl}-Me), 3.38 (s, 3H, 2_{Chl}-Me), 3.13 (s, 3H, 3^2_{Bchl}-Me), 2.82 (s, 3H, 7_{Chl}-Me), 2.52 (m, 2H, 1H from 17^2_{Bchl}-CH_2 and 1H from 17^2_{Chl}-CH_2), 2.31 (m, 2H, 1H from 17^2_{Bchl}-CH_2 and 1H from 8_{Bchl}-CH_2), 2.24 (m, 3H, 1H from 17^1_{Bchl}-CH_2 and 2H from NCH_2CH_2), 2.10 (m, 1H, 8_{Bchl}-CH_2), 2.05 (m, 5H, 4H from $NCH_2CH_2CH_2CH_2$ and 1H from 17^2_{Chl}-CH_2), 1.85 (m, 1H, 17^1_{Bchl}-CH_2), 1.73 (d, 3H, 7_{Bchl}-Me, *J* 7.6 Hz), 1.74 (br., 5H, 3H from 18_{Chl}-Me and 2H from 17^1_{Chl}), 1.64 (t, 3H, 8_{Chl}-Me, *J* 7.6 Hz), 1.61 (d, 3H, 18_{Bchl}-Me, *J* 7.2 Hz), 1.09 (t, 3H, 8_{Bchl}-Me, *J* 8 Hz), –0.51 (H, N_{BchlH}), –0.74 (H, N_{BchlH}).

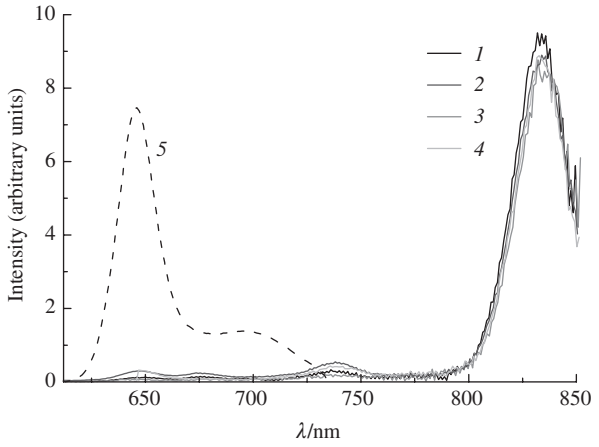


Figure 2 Fluorescence spectra of the Chl–Bchl heterodimer **5b** and chlorin **3b** in benzene upon excitation by different wavelengths: (*1*) 365, (*2*) 414, (*3*) 545 and (*4*) 635 nm; (*5*) fluorescence spectrum of chlorin **3b**, excitation wavelength of 414 nm.

chlorin; it is noteworthy that the absorption bands of chlorin in the dimer and in an isolated state are virtually the same.

Figure 2 shows that the main maximum of the dimer fluorescence spectrum obtained upon excitation at wavelengths corresponding to the absorption bands of chlorin and bacteriochlorin always matched the bacteriochlorin fluorescence maximum (836 nm) and differed considerably from that of chlorin (647 nm). This suggests that the excited singlet states of chlorin molecules populated due to chlorin photoexcitation, efficiently (with about 100% yield) transfer their energy to bacteriochlorin, presumably by the Förster resonance mechanism. It is known that the critical distance between chlorin chromophores corresponding to this mechanism of energy transfer is ≈ 50 Å.[18] The distance between the macrocycles in the dimer corresponding to the spacer (six C–C bonds) is about 10 Å, which obviously agrees with the high yield of energy transfer.

Thus, we obtained a hitherto unknown chlorin–bacteriochlorin dimer containing a Zn^{II}-complex of a chlorin e_6 derivative covalently bound with cycloimide of bacteriochlorin *p via* a spacer group. The electron excitation energy of chlorin in the dimer totally migrates to bacteriochlorin. The properties discovered, alongside with the intense absorption in the near-IR region, make it possible to consider dimers of this kind as promising sensitizers for the diagnostics and treatment of malignant neoplasms.

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