Synthesis of chlorin e_6 amide derivatives

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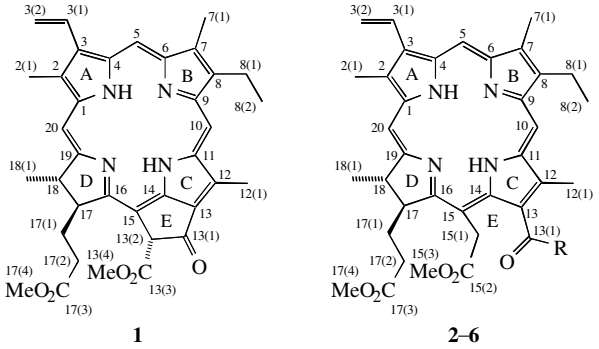
The reactions of methylpheophorbide *a* with primary and secondary amines have been investigated as a means for the synthesis of sensitizers used in the photodynamic therapy of tumors.

It was found earlier that the exo cycle E of methylpheophorbide *a* **1** can be easily opened by simple primary amines (methylamine[1] and ethylamine[2]) with the formation of amides **2** and **3** (Figure 1), respectively. The analogous interaction of **1** with secondary amines has not been reported, although it is interesting for the synthesis of sensitizers for the photodynamic therapy of tumors.[3,4]

It is well known that the insertion of a hydroxyl group increases the hydrophilicity of porphyrin and the selectivity of its accumulation in tumors.[3] Nucleophilic substitution with ethanolamine at C-13(1) in the exo cycle E of **1** may be a convenient method for the insertion of a hydroxyl group into the porphyrin molecule. In this work, secondary and tertiary chlorin e_6 amides **4**–**6** (Figure 1) were synthesised using the reaction of nucleophilic substitution at C-13(1) in the exo cycle E of **1**.†

Chlorin e_6 13(1)-*N*-(2-hydroxyethyl)amide-15(2),17(3)-dimethyl ester **4**‡ was prepared by the treatment of **1** with ethanolamine in chloroform at room temperature. Valency vibration band of C=O 13(1) keto group is absent from the IR spectrum of the substance obtained and bands 'amide-I' (at 1638 cm^{-1}), 'amide-II' (at 1526 cm^{-1}), valency vibration band of amide N–H (weak band at 3100 cm^{-1}) are present in this spectrum. The



1 **2–6**

1 Methylpheophorbide *a*
2 Chlorin e_6 13(1)-*N*-methylamide-15(2),17(3)-dimethyl ester: R = NHMe
3 Chlorin e_6 13(1)-*N*-ethylamide-15(2),17(3)-dimethyl ester: R=NHEt
4 Chlorin e_6 13(1)-*N*-(2-hydroxyethyl)amide-15(2),17(3)-dimethyl ester: $R=NHCH_2CH_2OH$
5 Chlorin e_6 13(1)-*N*,*N*-dimethylamide-15(2),17(3)-dimethyl ester: $R=NMe_2$
6 Chlorin e_6 13(1)-morpholinoamide-15(2),17(3)-dimethyl ester, numbering:

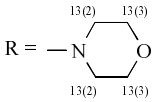


Figure 1 Chlorophyll derivatives.

^{1}H NMR spectrum shows a triplet of amide group NH proton (at 6.88 ppm) and doublets of 15(1)-methylene group protons [5.58 (d, 1H, *J* 18.4 Hz) and 5.31 (d, 1H, 18.4 Hz)]. The singlets of 5-, 10- and 20-protons in the amide spectrum are downfield shifted with respect to analogous signals of methylpheophorbide *a* spectrum, and a difference between their chemical shifts is smaller. The downfield shifts of 5-, 10- and 20-proton signals and a decrease of the chemical shift difference between 5- and 10-protons may be explained by an increase in 'ring current' and the leveling of this proton shielding caused by exo ring opening.

The interaction of **1** with secondary amines (dimethylamine, morpholine) was carried out by the same way. The structures of tertiary amides **5**§ and **6**¶ were determined by IR and NMR spectroscopy. The 'amide-I' bond in IR spectra of compounds obtained and 15(1)-methylene group signals in ^{1}H NMR spectra show that exo cycle E recovering and amide formation occur. An NMR and HPLC study of compounds **5** and **6** shows that each of these substances exists as two isomers in the ratio 2:1. We suppose that the 13(1)-amide group and a chlorin ring are in different planes, and tertiary amide isomers differ in the 13(1)-amide group position relatively to a chlorin ring. The ^{1}H and ^{13}C NMR spectra of the tertiary amides should be interpreted as a superposition of the spectra of two isomers ('double set of signals'). Each of superposed spectra has the same amount of signals (for ^{1}H and ^{13}C NMR spectra) of the same multiplicity (for ^{1}H NMR spectra). The intensity ratio between isomer signals in ^{1}H NMR spectra of both tertiary amides is 2:1. HPLC data are consistent with NMR data. The HPLC of **5** shows two peaks in a ratio of 2:1 (by intensity).

† ^{1}H and ^{13}C NMR spectra were obtained in deuterochloroform solutions at 300 and 75 MHz using a Bruker AM-300 spectrometer. Signals were assigned by a comparison with the spectra of chlorin e_6 13(1)-*N*-ethylamide-15(2),17(3)-dimethyl ester **3**.[2] IR spectra were obtained in KBr pellets on a Specord M80 spectrometer. UV-VIS spectra were obtained in chloroform solutions using a Lambda 20 spectrometer (Perkin–Elmer) in a range of 350–750 nm. HPLC analysis was carried out on a 'Milikhrom 1' chromatograph (column 2×64 mm; Silasorb 600, 5.0 μm). Benzene–ethyl acetate in a ratio of 7:5 was used as an eluent for the analysis of amide **5**. Silica gel (La Chema, 40–100 mesh) was used for column chromatography.

General procedure for the synthesis of chlorin e_6 13(1)-amides-15(2),17(3)-dimethyl ester. A solution of **1** in chloroform (3 ml) was stirred with an amine (0.3 ml; for dimethylamine, 0.5 ml of a 33% aqueous solution) at room temperature until the absence of the starting material (TLC). The reaction mixture was diluted with chloroform (50 ml), washed with water (3×100 ml), dried (Na_2SO_4) and evaporated to dryness at 30–40 °C under reduced pressure. The product was purified by column chromatography on silica gel (eluent: tetrachloromethane–acetone) and reprecipitated from chloroform–pentane.

‡ *Chlorin e_6 13(1)-*N*-(2-hydroxyethyl)amide-15(2),17(3)-dimethyl ester.* Compound **4** (222 mg, 63%) was obtained from 322 mg of **1**. Amide **4** was eluted with CCl_4–acetone (3:1, v/v). ^{1}H NMR, δ: 9.69 (s, 1H, 10-H), 9.64 (s, 1H, 5-H), 8.81 (s, 1H, 20-H), 8.10 [dd, 1H, 3(1)-H, *J* 17.7 and 11.6 Hz], 6.37 [dd, 1H, 3(2)-H (*trans*) *J* 17.7 and 1.4 Hz], 6.15 [dd, 1H, 3(2)-H (*cis*), *J* 11.6 and 1.4 Hz], 6.88 [br. t, 1H, 13(1)-NH (amide), *J* 5.7 Hz], 5.58 [d, 1H, 15(1)-CH_2(B), *J* 18.4 Hz], 5.31 [d, 1H, 15(1)-CH_2(A), *J* 18.4 Hz], 4.47 (m, 1H, 18-H), 4.40 (m, 1H, 17-H), 3.75 [s, 3H, 15(3)-Me], 3.61 [s, 3H, 17(4)-Me], 3.57 [s, 3H, 12(1)-Me], 3.50 [s, 3H, 1(1)-Me], 3.31 [s, 3H, 7(1)-Me], 3.81 [q, 2H, 8(1)-CH_2, *J* 7.5 Hz], 2.2–2.6 [m, 4H, 17(1)-CH_2, 17(2)-CH_2], 1.71 [d, 3H, 18(1)-Me, *J* 7.0 Hz], 1.72 [t, 3H, 8(2)-Me, *J* 7.5 Hz], 3.80–3.95 [m, 2H, 13(2)-CH_2], 4.02 [t, 2H, 13(3)-CH_2, *J* 5 Hz]. ^{13}C NMR, δ: 174.37, 173.53, 170.48, 168.94, 166.63, 154.34, 149.06, 144.82, 138.99, 136.10, 134.92, 134.61, 130.26, 129.77, 129.43, 127.72, 121.68, 102.02, 101.47, 98.84, 93.65, 62.09, 53.03, 52.27, 51.59, 49.18, 43.55, 37.98, 31.06, 29.72, 17.68, 12.13, 11.99, 11.32. IR (KBr, ν/cm^{-1}): 1740 (ester $\nu_{C=O}$), 1638 ('amide-I'), 1610 ('chlorin band'), 1526 ('amide-II'). UV-VIS [$CHCl_3$, λ/nm (lg ε)]: 663.7 (4.76), 608.1 (3.76), 557.9 (3.34), 529.0 (3.69), 500.5 (4.21), 401.6 (5.13).

It is possible that different positions of the 13(1)-amide group relatively to the chlorin ring influence the electronic surrounding of nearest protons and, therefore, the chemical shifts of their signals. For example, the greatest chemical shift difference of proton signals between isomers is observed for 15(1)-CH_2 protons. It indicates different influences of the amide group on the electronic surroundings of these protons at different positions relatively to the chlorin ring.

Longwave bonds in the electron spectra of all amides are in the range 663–664 nm. Thus, these compounds should be interesting as potential sensitizers for the photodynamic therapy.

§ *Chlorin e_6 13(1)*-N,N-*dimethylamide-15(2),17(3)-dimethyl ester*. Compound **5** (35 mg, 54%) was obtained from 60 mg of **1**. The amide was eluted with CCl_4–acetone (10:1, v/v). ^{1}H NMR, δ: major isomer: 9.75 (s, 1H, 10-H), 9.71 (s, 1H, 5-H), 8.88 (s, 1H, 20-H), 8.15 (dd, 1H, *J* 17.9 and 11.5 Hz), 6.40 [dd, 1H, 3(2)-H (*trans*), *J* 1.8 and 19.6 Hz], 6.18 [dd, 1H, 3(2)-H (*cis*), *J* 1.6 and 11.5 Hz], 5.88 [d, 1H, 15(1)-CH(B), *J* 19 Hz], 5.08 [d, 1H, 15(1)-CH(A), *J* 18.9 Hz], 4.4–4.6 (m, 2H, 18-H, 17-H), 3.84 [s, 3H, 15(3)-Me], 3.68 [s, 3H, 17(4)-Me], 2.78 [s, 3H, 13(1)-NMe_2], 3.4–4.0 [m, 14H, 12(1)-Me, 1(1)-Me, 7(1)-Me, 13(1)-NMe_2, 8(1)-CH_2], 2.1–2.7 [m, 4H, 17(1)-CH_2, 17(2)-CH_2], 1.75 [m, 6H, 18(1)-Me, 8(2)-Me]; minor isomer: 9.73 (s, 1H, 10-H), 9.68 (s, 1H, 5-H), 8.83 (s, 1H, 20-H), 8.13 (dd, 1H, *J* 17.7 and 11.5 Hz), 6.40 [dd, 1H, 3(2)-H (*trans*), *J* 1.8 and 19.6 Hz], 6.18 [dd, 1H, 3(2)-H (*cis*), *J* 1.6 and 11.5 Hz], 5.73 [d, 1H, 15(1)-CH(B), *J* 19.3 Hz], 5.16 [d, 1H, 15(1)-CH(A), *J* 19.2 Hz], 4.4–4.6 (m, 2H, 18-H, 17-H), 3.80 [s, 3H, 15(3)-Me], 3.70 [s, 3H, 17(4)-Me], 3.13 [s, 3H, 13(1)-NMe_2], 3.4–4.0 [m, 14H, 12(1)-Me, 1(1)-Me, 7(1)-Me, 13(1)-NMe_2, 8(1)-CH_2], 2.1–2.7 [m, 4H, 17(1)-CH_2, 17(2)-CH_2], 1.75 [m, 6H, 18(1)-Me, 8(2)-Me]. ^{13}C NMR, δ: major isomer: 173.45, 173.29, 170.64, 168.70, 167.28, 154.20, 149.32, 144.74, 138.79, 136.27, 134.76, 134.62, 133.77, 130.42, 130.09, 129.66, 127.09, 121.60, 102.46, 101.01, 98.91, 93.88, 52.96, 49.18, 51.92, 51.77, 37.04, 35.31, 31.36, 29.87, 23.10, 19.80, 17.58, 12.24, 11.86, 11.35; minor isomer: 173.45, 173.29, 170.19, 168.96, 166.53, 154.20, 149.08, 144.74, 138.79, 136.18, 135.19, 134.52, 133.77, 130.42, 130.20, 129.66, 127.09, 121.60, 102.77, 101.01, 98.91, 93.65, 53.75, 49.53, 52.08, 51.77, 37.73, 35.27, 31.93, 29.87, 23.19, 19.80, 17.58, 12.24, 12.00, 11.35. IR (KBr, ν/cm^{-1}): 1746 (ester $\nu_{C=O}$), 1632 ('amide-I'), 1613 ('chlorin band'). UV-VIS [$CHCl_3$, λ/nm (lg ε)]: 663.7 (4.70), 608.0 (3.62), 559.7 (2.98), 528.7 (3.47), 500.6 (4.13), 402.1 (5.19).

¶ *Chlorin e_6 13(1)-morpholinoamide-15(2),17(3)-dimethyl ester*. Compound **6** (24 mg, 42%) was obtained from 50 mg of **1**. The amide was eluted with CCl_4–acetone (4:1, v/v). ^{1}H NMR, δ: major isomer: 9.72 (s, 1H, 10-H), 9.67 (s, 1H, 5-H), 8.86 (s, 1H, 20-H), 8.11 (dd, 1H, *J* 17.0 and 11.6 Hz), 6.38 [dd, 1H, 3(2)-H (*trans*), *J* 17.9 and 1.5 Hz], 6.16 [dd, 1H, 3(2)-H (*cis*), *J* 11.6 and 1.6 Hz], 5.81 [d, 1H, 15(1)-CH(B), *J* 19.0 Hz], 5.09 [d, 1H, 15(1)-CH(A), *J* 19.0 Hz], 4.42–4.65 (m, 2H, 18-H, 17-H), 3.57 [s, 3H, 15(3)-Me], 3.66 [s, 3H, 17(4)-Me], 3.88 [s, 3H, 12(1)-Me], 3.51 [s, 3H, 1(1)-Me], 3.34 [s, 3H, 7(1)-Me], 3.7–4.2 [m, 10H, 8(1)-CH_2, 13(2)-CH_2, 13(3)-CH_2], 2.2–2.7 (m, 4H), 1.6–1.8 [m, 6H, 18(1)-Me, 8(2)-Me]; minor isomer: 9.69 (s, 1H, 10-H), 9.64 (s, 1H, 5-H), 8.81 (s, 1H, 20-H), 8.11 [dd, 1H, 3(2)-H], 6.38 [dd, 1H, 3(2)-H (*trans*), *J* 17.9 and 1.5 Hz], 6.16 [dd, 1H, 3(2)-H (*cis*), *J* 11.6 and 1.6 Hz], 5.52 [d, 1H, 15(1)-CH(B), *J* 19.0 Hz], 5.25 [d, 1H, 15(1)-CH(A), *J* 19.0 Hz], 4.42–4.65 (m, 2H, 18-H, 17-H), 3.54 [s, 3H, 15(3)-Me], 3.62 [s, 3H, 17(4)-Me], 3.83 [s, 3H, 12(1)-Me], 3.50 [s, 3H, 1(1)-Me], 3.33 [s, 3H, 7(1)-Me], 3.7–4.2 [m, 10H, 8(1)-CH_2, 13(2)-CH_2, 13(3)-CH_2], 2.2–2.7 [m, 4H, 17(1)-CH_2, 17(2)-CH_2], 1.6–1.8 [m, 6H, 18(1)-Me, 8(2)-Me]. ^{13}C NMR, δ: major isomer: 173.45, 173.29, 169.24, 168.78, 167.16, 154.39, 149.00, 144.73, 138.86, 136.15, 134.89, 134.55, 134.40, 133.60, 130.22, 130.03, 129.47, 125.54, 121.64, 102.23, 101.01, 98.78, 93.87, 67.00, 52.10, 51.59, 49.43, 42.51, 37.08, 31.19, 30.87, 29.65, 19.67, 17.67, 12.14, 12.11, 11.33; minor isomer: 173.61, 173.04, 169.00, 168.68, 166.55, 154.39, 149.19, 144.78, 138.93, 136.22, 134.97, 135.12, 134.61, 133.43, 130.28, 129.83, 129.47, 125.74, 121.64, 102.29, 101.22, 98.78, 93.65, 66.84, 52.24, 51.59, 49.13, 42.43, 37.79, 31.19, 30.87, 29.76, 19.67, 17.67, 12.22, 12.11, 11.33. IR (KBr, ν/cm^{-1}): 1742 (ester $\nu_{C=O}$), 1636 ('amide-I'), 1608 ('chlorin band'). UV-VIS [$CHCl_3$, λ/nm (lg ε)]: 663 (4.73), 608.7 (3.74), 557.1 (3.41), 528.5 (3.68), 500.5 (4.19), 402.2 (5.21).

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