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Synthesis of Amide Derivatives of Chlorin e_6

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Abstract—A number of secondary and tertiary chlorin e_6-13-amides were synthesized in high yield by the action of primary and secondary amines on methylpheophorbide *a* under mild conditions. Unlike the secondary amides, tertiary 13-amides were shown to exist as two stereoisomers differing by orientation of the amide group plane with respect to the macroring. The reaction of methylpheophorbide *a* with 2-aminoethanol gave chlorin e_6 derivatives containing one, two, and three hydroxy groups.

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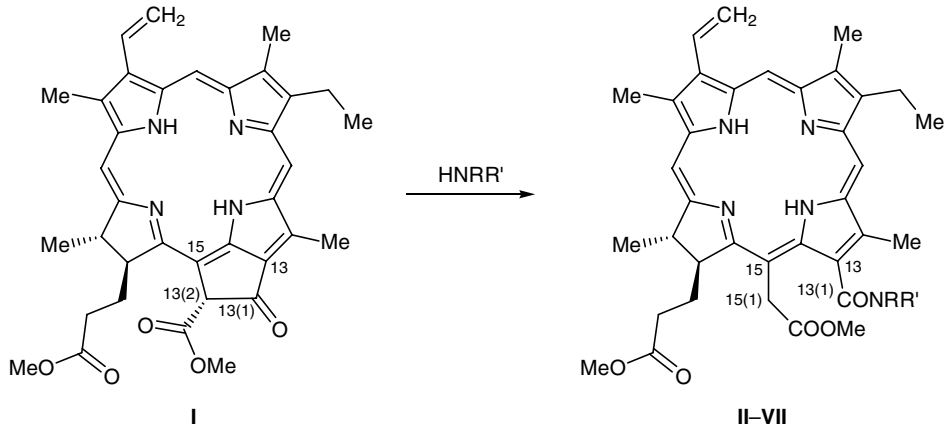
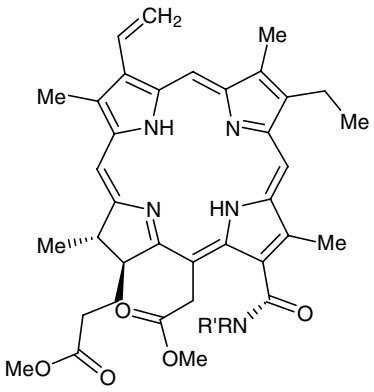
Chemical transformations of plant porphyrins attract interest from the viewpoint of synthesis of biologically active compounds, e.g., sensitizers for photodynamic therapy of cancer [1–5]. In particular, chlorophyll derivatives are active as photosensitizers [3–5]. Opening of the exo ring in methylpheophorbide *a* (**I**) by the action of amines could serve as a convenient method for the introduction of various groups at the periphery of porphyrin molecules. It is known that the exo ring in **I** is readily cleaved by the action of lower primary amines such as methyl- [6] and ethylamine [7] to give the corresponding amides **II** and **III** (Scheme 1). By varying the substituent on the amine nitrogen atom, the series of available amides could be extended; as a result, the hydrophilicity of chlorophyll derivatives thus obtained and their tropism for various malignant tumors could be controlled. Introduction of a hydroxy group enhances the hydrophilicity of porphyrins and selectivity of their accumulation in tumors [3]. An analogous effect can be produced by introduction of an amino group, for the latter, like hydroxy group, tends to form hydrogen bonds. Thus reactions of **I** with primary and secondary amines could give rise to various biologically active amide derivatives.

Scheme 1.

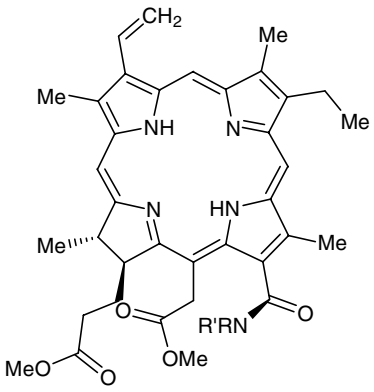


I **II–VII**

II, R = Me, R' = H; **III**, R = Et, R' = H; **IV**, R = $NH_2CH_2CH_2$, R' = H; **V**, R = $HOCH_2CH_2$, R' = H; **VI**, R = R' = Me; **VII**, R = R' = Et; **VIII**, RR'N = morpholino.



A



B

In the present work we synthesized a number of secondary and tertiary chlorin e_6-13-amides **IV**–**VIII** by the action of primary and secondary amines on the β-keto ester moiety in molecule **I** (Scheme 1) and examined side processes accompanying the formation of the target products.

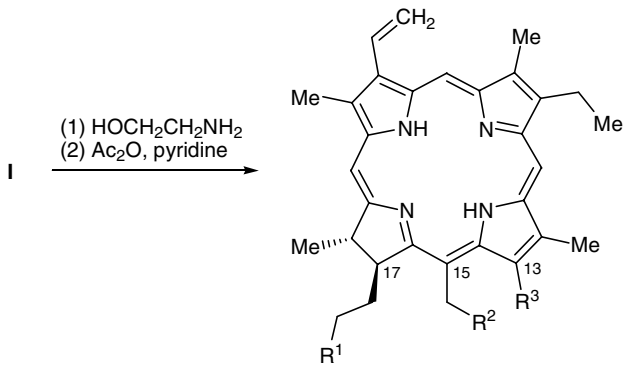
Reactions of **I** with primary and secondary amines can be regarded as nucleophilic substitution at the carbonyl carbon atom in the 13(1)-position of the exo ring. As nucleophiles we used ethane-1,2-diamine, 2-aminoethanol, dimethylamine, diethylamine, and morpholine. The reactions occurred under mild conditions, and the yield of the resulting amides ranged from 50 to 60%. The IR spectra of the products lacked absorption band due to ketone carbonyl $C^{13(1)}$=O, which is typical of initial compound **I**, but amide I (**IV**–**VIII**) and amide II bands appeared (**IV**, **V**). The ^{1}H NMR spectra of **IV**–**VIII** contain no singlet from proton in the 13(2)-position of the exo ring, but *AB* spin system was observed due to protons of the methylene group on C^{15}, which was formed as a result of opening of the exo ring (Figs. 1, 2). In the ^{1}H NMR spectra of secondary amides **IV** and **V**, broadened triplets from the NH protons were also present. Protons in the amine residues gave rise to the corresponding signals in the ^{1}H and ^{13}C NMR spectra. All amides **IV**–**VIII** displayed in the electronic absorption spectra a band in the region λ 660–664 nm (band *I*) due to chlorin chromophore.

NMR and HPLC studies of tertiary amides **VI**–**VIII** showed that each of these compounds is a mixture of two isomers at a ratio of ~2 : 1 (two peaks with an intensity ratio of 2 : 1 were observed on the chromatograms of amides **VI** and **VII**). Their ^{1}H and ^{13}C NMR spectra may be interpreted as superpositions of the spectra of two isomers (double set of signals; see Figs. 1, 2). Each of the superimposed spectra has the same number of signals (^{1}H and ^{13}C) with the same multiplicity (^{1}H), and the signal intensity ratio is about 2 : 1. These data may be rationalized as follows. The 13-amide group and the chlorin macroring lie in different planes, and isomers **A** and **B** differ by orientation of the 13-amide group relative to the chlorin ring plane. Different arrangement of the amide group with respect to the macroring plane should be reflected in the chemical shifts of the neighboring protons; thus the maximal difference in the chemical shifts is observed for the methylene protons on C^{15}.

Study of the composition of products formed in the reaction of methylpheophorbide *a* (**I**) with amines showed that nucleophilic substitution at the 13(1)-carbonyl carbon atom is accompanied by amidation of the ester groups and isomerization. These side processes were examined in more detail. The amidation products formed in the reaction of methylpheophorbide *a* (**I**) with 2-aminoethanol, diamide **X** and triamide **XII**, were isolated by column chromatography on silica gel (Scheme 2). Their structure was proved by spectral methods and conversion into the corresponding acetates **XI** and **XIII**.

The IR spectrum of triamide **XII** contained no absorption assignable to stretching vibrations of ester carbonyl, but amide I and amide II bands were present at 1650 and 1550 cm^{-1}. In the ^{1}H NMR spectrum of triacetate **XIII** derived from triamide **XII** we observed three three-proton singlets at δ 2.15, 1.89, and 1.52 ppm, corresponding to methyl protons in the acetoxy groups; these data confirm the presence of three hydroxy groups in initial triamide **XII**. Comparison of the ^{1}H NMR spectrum of **XIII** with the spectrum of monoamide acetate **IX** showed that both ester groups in **I** underwent amidation: no signals from the 17(4)- and 15(3)-methyl groups were present in the spectrum of **XIII**. The amide NH protons in molecule **XIII** gave

Scheme 2.



IX–XIII

IX, R^1 = MeOCO, R^2 = $MeOCOCH_2$, R^3 = $AcOCH_2CH_2NHCO$; **X**, $R^1 = R^3$ = $HOCH_2CH_2NHCO$, R^2 = $MeOCOCH_2$; **XI**, $R^1 = R^3$ = $AcOCH_2CH_2NHCO$, R^2 = $MeOCOCH_2$; **XII**, $R^1 = R^2 = R^3$ = $HOCH_2CH_2NHCO$; **XIII**, $R^1 = R^2 = R^3$ = $AcOCH_2CH_2NHCO$.

three broadened one-proton triplets at δ 7.48, 6.90, and 5.29 ppm. The ^{13}C NMR data were consistent with the ^1H NMR spectra. The ^{13}C NMR spectrum of triacetate **XIII** lacked signals from the ester methyl groups (δ_C 51.59 and 52.27 ppm in the spectrum of **IX**). Unlike compound **IX**, the ^{13}C NMR spectrum of **XIII** contained signals from three 2-acetoxyethylamino fragments, δ_C, ppm: 39.07, 39.96, 40.35 (NCH_2); 63.17, 63.31, 63.74 (OCH_2). The corresponding signals in the spectrum of **IX** appeared at δ_C 43.55 (NCH_2) and 62.09 ppm (OCH_2). In addition, three upfield signals from the methyl groups in the acetyl fragments were present in the spectrum of **XIII** (δ_C 20.78, 21.14, and 21.40 ppm) together with three downfield signals from the acetyl carbonyl carbon atoms; the amide carbonyl carbon atoms in **XIII** and ester carbonyl carbon atoms in **XI** were characterized by similar chemical shifts.

Diamide **X** displayed in the IR spectrum an absorption band due to stretching vibrations of the ester carbonyl group, but its intensity was considerably lower than in the spectrum of monoamide **V**. On the other hand, the intensity of bands corresponding to vibrations of the amide groups was appreciably higher, as compared to **V**. The diamide structure of **X** was also confirmed by the mass and ^1H and ^{13}C NMR spectra of the corresponding acetylated product, compound **XI**. The mass spectrum of **XI** contained the molecular ion peak with *m*/*z* 780. In the ^1H NMR spectrum of **XI** we observed two three-proton singlets at δ 2.12 and 1.75 ppm from the acetyl protons and two broadened one-proton triplets at δ 6.76 and 5.04 ppm due to NH protons. Diamide diacetate **XI** showed in the ^{13}C NMR spectrum two sets of signals from two 2-acetoxyethyl groups. The absence of a three-proton singlet at

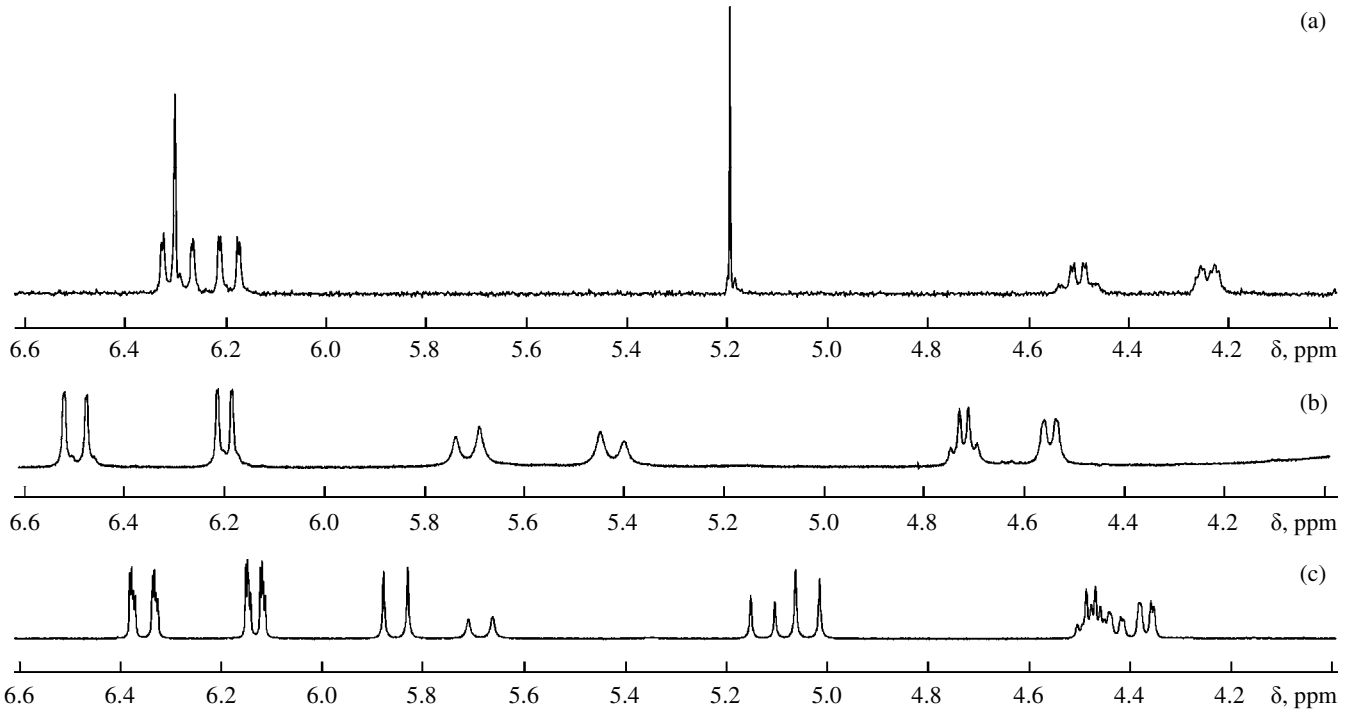


Fig. 1. ^1H NMR spectra in the region δ 4.0–6.6 ppm of (a) methylpheophorbide *a* (**I**) ($CDCl_3$, 300 MHz), (b) *N*-(2-aminoethyl)-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (**IV**) (DMF-d_7, 400 MHz), and (c) *N*,*N*-dimethyl-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (**VI**) ($CDCl_3$, 400 MHz).

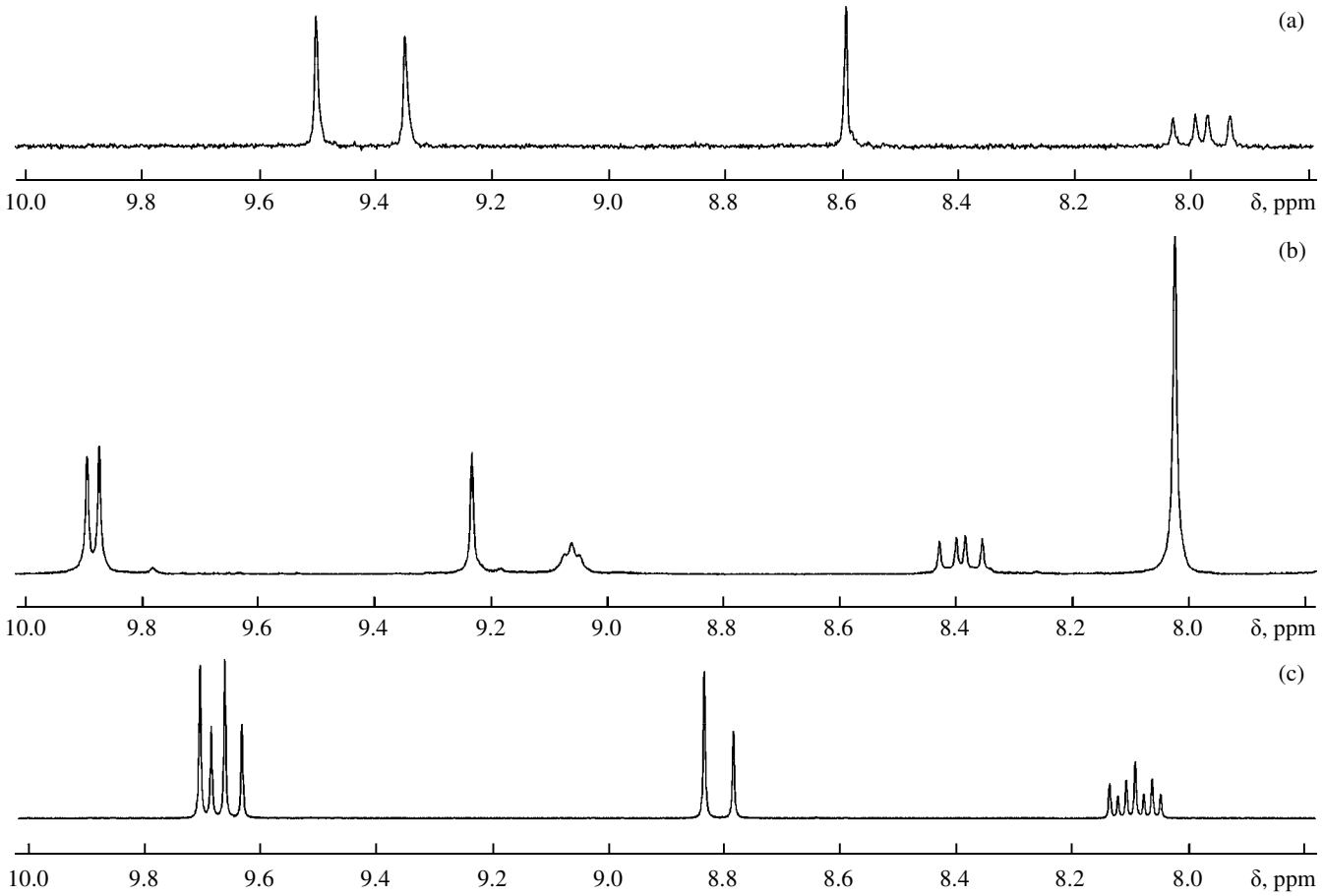


Fig. 2. ^{1}H NMR spectra in the resonance region of *meso* protons of (a) methylpheophorbide *a* (**I**) ($CDCl_3$, 300 MHz), (b) *N*-(2-aminoethyl)-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (**IV**) (DMF-d_7, 400 MHz), and (c) *N*,*N*-dimethyl-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (**VI**) ($CDCl_3$, 400 MHz).

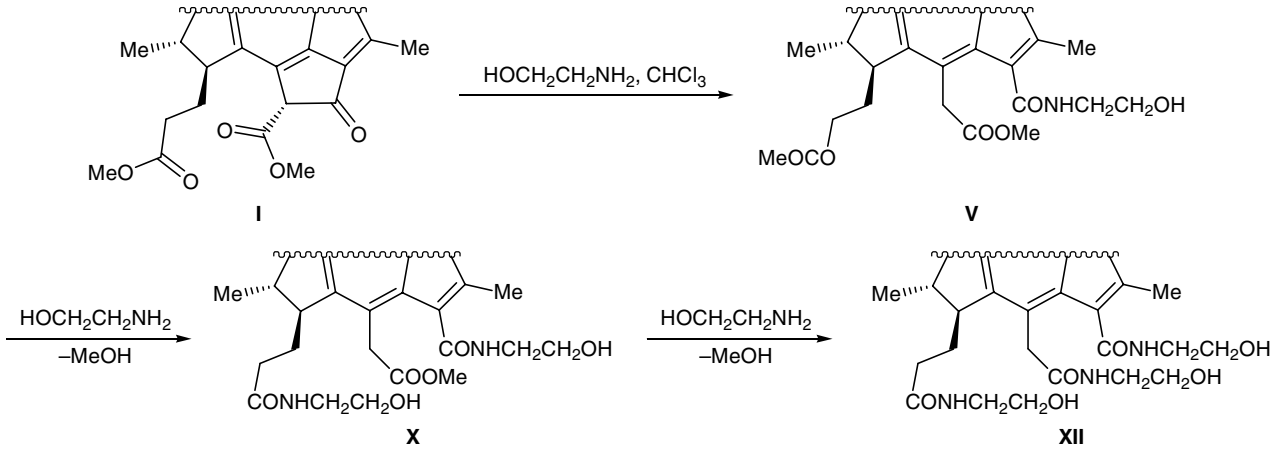
δ 3.60 ppm in the ^{1}H NMR spectrum of **XI** (this signal is observed in the spectrum of acetate **IX**; it belongs to the ester methyl group in the substituent on C^{17}) indicates that the amination occurred at the ester group in the propionate fragment attached to C^{17}.

Diamide **X** and triamide **XII** attract interest as potential sensitizers for photodynamic therapy. Therefore, we tried to optimize the procedures for their preparation from both monoamide **V** and directly from methylpheophorbide *a* (**I**). For this purpose, monoamide **V** was dissolved in pure 2-aminoethanol. After 6–8 h, the conversion of the initial compound was complete, and the product was diamide **X** containing a small impurity of triamide **XII**; after 60 h, compound **X** was completely converted into triamide **XII** (according to the TLC data). Methylpheophorbide *a* (**I**) is poorly soluble in 2-aminoethanol; therefore, the reaction was carried out in chloroform. The solvent was removed from the reaction mixture under reduced pressure, and the resulting monoamide **V** reacted with 2-aminoethanol (which was not distilled off) to give amides **X** and **XII** in a way similar to that described above. Thus the reaction of methylpheophorbide *a* (**I**) with 2-aminoethanol could give chlorin e_6 derivatives containing one, two, and three hydroxy groups.

Our results indicate that the reaction of methylpheophorbide *a* (**I**) with 2-aminoethanol begins with opening of the exo ring; next follows amidation of the ester groups in monoamide **V**, the propionate substituent on C^{17} reacting first; finally, the acetate moiety on C^{15} undergoes amidation (Scheme 3). No 15-amide-17-ester derivative was detected in the reaction mixture. The lower reactivity of the ester moiety on C^{15} is likely to originate from steric shielding of that group.

Ellsworth and Storm [6] reported on the different reactivities of methylpheophorbide *a* (**I**) and its diastereoisomer at the 13(2)-position, methylpheophorbide *a*' (**XIV**) (Scheme 4) in nucleophilic substitution at the $C^{13(1)}$ carbonyl carbon atom. Treatment of a mixture of isomers **I** and **XIV** with methylamine in THF

Scheme 3.



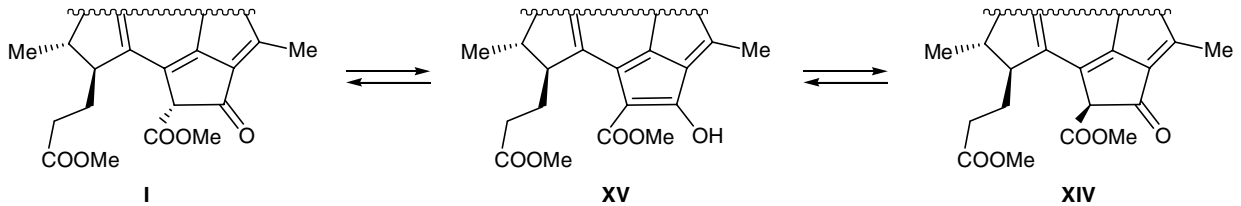
gave amide **II**, but the reaction occurred mainly with methylpheophorbide *a* (**I**), while diastereoisomer **XIV** was recovered from the reaction mixture. To compare the reactivity of isomers **I** and **XIV** under the amidation conditions, their ~1 : 1 mixture (HPLC) was brought into reactions with 2-aminoethanol, morpholine and dimethylamine [the conditions were the same as in the reactions with pure methylpheophorbide *a* (**I**)]. In all cases, no accumulation of methylpheophorbide *a*' (**XIV**) in the unreacted substrate was observed. According to the HPLC data, the reaction mixtures contained a small amount of isomer mixture **I/XIV** at a ratio of 4 : 1, regardless of whether pure methylpheophorbide *a* (**I**) or equimolar isomer mixture **I/XIV** was taken. The observed isomer ratio established in 5–10 min after the reaction started. The appearance of isomer **XIV** in the reactions of amines with pure methylpheophorbide *a* (**I**), as well as decrease in the fraction of methylpheophorbide *a*' (**XIV**) in the reactions with diastereoisomer mixture, may be rationalized in terms of reversible isomerization of **I** through intermediate enol **XV** (Scheme 4). An analogous pattern was observed in the synthesis of chlorophylls *a*' and *b*' [8, 9]: the isomerization occurred in the presence of triethylamine. Our results and published data suggest that the isomerization is catalyzed by the amines used as reactants.

Thus we observed no difference in the reactivities of methylpheophorbides *a* (**I**) and *a*' (**XIV**) under the amidation conditions. Even if such a difference exists, it is leveled due to reversible isomerization. Our results show that the reaction in chloroform can be performed with methylpheophorbide *a* (**I**) isolated from natural sources [which contains about 20% of methylpheophorbide *a*' (**XIV**), according to the HPLC data] without additional crystallization.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded from solutions in chloroform-*d* or DMF-d_7 on Bruker AM-300 and Bruker AMX-400 spectrometers (300 or 400 MHz for ^{1}H and 75 MHz for ^{13}C). Signals were assigned by comparing with the spectra of chlorin e_6 - 13(1)-*N*-ethylamide-15(2),17(3)-dimethyl ester (**III**) [7]. For tertiary amides, the spectra were recorded from isomer mixtures. The mass spectra were obtained on a Kratos MS-890 mass spectrometer. The IR spectra were recorded in KBr (~1–1.5-mg samples) on a Specord M-80 instrument. The electronic absorption spectra were measured from solutions in chloroform in the λ range from 350 to 750 nm on a Perkin–Elmer Lambda 20 spectrophotometer. HPLC analysis was performed on a Milikhrom 1 chromatograph equipped with a 2 × 64-mm column packed with Silasorb 600

Scheme 4.



(5.0 μm); detection at λ 360 nm; eluent benzene– ethyl acetate (7:5, by volume, for **VI**, **VII**; 10:1 for **I**, **XIV**). Silica gel L 40/100 μm was used for column chromatography.

Methylpheophorbid *a* (I) was obtained as described in [10] from lipophilic fraction of the extract isolated from *Serratula coronata L.* ^{1}H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: 9.48 s (1H, 10-H), 9.33 s (1H, 5-H), 8.58 s (1H, 20-H), 7.97 d.d [1H, 3(1)-H, *J* = 18.4, 11.5 Hz], 6.27 d.d [1H, *trans*-3(2)-H, *J* = 17.8, 1.4 Hz], 6.17 d.d [1H, *cis*-3(2)-H, *J* = 12.8, 1.4 Hz], 6.28 s [1H, 13(2)-H], 4.50 q.d (1H, 18-H, *J* = 8.8, 2.8 Hz), 4.22 m (1H, 17-H), 3.91 s [3H, 13(2)-$COOCH_3$], 3.65 s [3H, 12(1)-H], 3.61 q [2H, 8(1)-H, *J* = 8.1 Hz], 3.59 s [3H, 17(3)-OCH_3], 3.40 s [3H, 2(1)-H], 3.19 s [3H, 7(1)-H], 2.39–2.20 m [4H, 17(1)-H, 17(2)-H], 1.84 d [3H, 18(1)-H, *J* = 7.2 Hz], 1.68 t [3H, 8(2)-H_3, *J* = 7.6 Hz], 0.51 br.s (*I*-NH), –1.67 br.s (*III*-NH). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 11.27, 12.18, 12.18, 17.48, 19.48, 23.17, 29.93, 31.12, 50.18, 51.18, 51.76, 52.95, 64.79, 93.19, 97.58, 104.43, 105.26, 122.85, 129.01, 129.19, 131.29, 136.22, 136.31, 136.56, 137.99, 142.12, 145.27, 149.72, 151.04, 155.69, 161.27, 169.67, 172.25, 173.44, 189.72.

15(2),17(3)-Dimethoxychlorin e_6-13(1)-amides (*general procedure*). A solution of 30 to 350 mg of methylpheophorbide *a* (**I**) in 3 ml chloroform and 0.3 ml of the corresponding amine (0.5 ml of 33% aqueous dimethylamine) was stirred at room temperature until initial compound **I** disappeared (TLC). The mixture was diluted with 50 ml of chloroform, washed with water (3×100 ml), dried over Na_2SO_4, and evaporated under reduced pressure at 30–40°C. The product was purified by column chromatography on silica gel using CCl_4–acetone as eluent and subsequent reprecipitation from chloroform with pentane.

***N*-(2-Aminoethyl)-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (IV)** was synthesized from 65 mg of methylpheophorbide *a* (**I**). The product was isolated using chloroform–methanol (9:1, by volume) as eluent. Yield 41 mg (69%). IR spectrum, ν, cm^{-1}: 1740 (C=O, ester), 1640 (C=O, amide I), 1609 (chlorin), 1525 (δNH, amide II). Electronic absorption spectrum, λ_{max}, nm (logε): 663.36 (4.74), 607.82 (3.74), 557.48 (3.33), 529.04 (3.67), 500.69 (4.19), 402.92 (5.18). ^{1}H NMR spectrum (DMF-d_7, 400 MHz), δ, ppm: 9.89 s (1H, 10-H), 9.87 s (1H, 5-H), 9.23 s (1H, 20-H), 9.06 br.t [1H, 13(1)-NH, *J* = 5.7 Hz], 8.39 d.d [1H, 3(1)-H, *J* = 17.6, 11.6 Hz], 6.49 d.d [1H, *trans*-3(2)-H, *J* = 18.0, 1.2 Hz], 6.20 d.d [1H, *cis*-3(2)-H, *J* = 11.6, 1.2 Hz], 5.71 d and 5.42 d [1H each, 15(1)-H, *J* = 19.0 Hz], 4.72 br.q (1H, 18-H, *J* = 7.2 Hz), 4.34 m (1H, 17-H), 3.8–4.0 m [4H, 13(2)-H, 13(3)-H], 3.78 s [3H, 15(3)-H], 3.64 s [3H, 17(4)-H], 3.60 s [3H, 12(1)-H], 3.59 s [3H, 2(1)-H], 3.37 s [3H, 7(1)-H], 3.7–3.8 m [2H, 8(1)-H], 3.24 br.t [1H, 13(3)-NH_2, *J* = 6.2 Hz], 2.2–2.7 m [2H, 17(2)-H], 1.6–1.8 m [2H, 17(1)-H], 1.70–1.73 m [6H, 8(2)-H, 18(1)-H], –1.61 br.s (1H, *I*-NH), –1.93 br.s (1H, *III*-NH). Mass spectrum: *m*/*z* 667 $[M]^+$.

***N*-(2-Hydroxyethyl)-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (V).** was synthesized from 323 mg of methylpheophorbide *a* (**I**); the product was isolated using CCl_4–acetone (3:1, by volume) as eluent. Yield 223 mg (63%). IR spectrum, ν, cm^{-1}: 1740 (C=O, ester), 1638 (C=O, amide I), 1610 (chlorin), 1526 (δNH, amide II). Electronic absorption spectrum, λ_{max}, nm (logε): 663.66 (4.76), 608.14 (3.76), 557.90 (3.34), 529.02 (3.69), 500.53 (4.21), 401.55 (5.13). ^{1}H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: 9.69 s (1H, 10-H), 9.64 s (1H, 5-H), 8.81 s (1H, 20-H), 8.10 d.d [1H, 3(1)-H, *J* = 17.7, 11.6 Hz], 6.37 d.d [1H, *trans*-3(2)-H, *J* = 17.7, 1.4 Hz], 6.15 d.d [1H, *cis*-3(2)-H, *J* = 11.6, 1.4 Hz], 6.88 br.t [1H, 13(1)-NH, *J* = 5.7 Hz], 5.58 d and 5.31 d [1H each, 15(1)-H, *J* = 18.4 Hz], 4.47 m (1H, 18-H), 4.40 m (1H, 17-H); 3.80–3.95 m and 4.02 t [2H each, 13(2)-H, 13(3)-H, *J* = 5.0 Hz], 3.75 s [3H, 15(3)-H], 3.61 s [3H, 17(4)-CH_3], 3.57 s [3H, 12(1)-H], 3.50 s [3H, 1(1)-H], 3.31 s [3H, 7(1)-H], 3.81 q [2H, 8(1)-H, *J* = 7.5 Hz], 2.2–2.6 m [4H, 17(1)-H, 17(2)-H], 1.71 d [3H, 18(1)-H, *J* = 7.0 Hz], 1.72 t [3H, 8(2)-H, *J* = 7.5 Hz]. ^{13}C NMR spectrum ($CDCl_3$, 75 MHz), δ_C, ppm: 174.37, 173.53, 170.48, 168.94, 166.63, 154.34, 149.06, 144.82, 138.99, 136.10, 134.92, 134.6, 130.26, 129.77, 129.43, 127.72, 121.68, 102.02, 101.47, 98.84, 93.65, 62.09, 53.03, 49.18, 52.27, 51.59, 43.55, 37.98, 31.06, 29.72, 17.68, 12.13, 11.99, 11.32. Mass spectrum: *m*/*z* 666 $[M]^+$.

***N*,*N*-Dimethyl-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (VI)** was synthesized from 60 mg of methylpheophorbide *a* (**I**); the product was isolated using CCl_4–acetone (10:1, by volume) as eluent. Yield 35 mg (54%) IR spectrum, ν, cm^{-1}: 1746 (C=O, ester), 1632 (C=O, amide I), 1613 (chlorin). Electronic absorption spectrum, λ_{max}, nm (logε): 663.72 (4.70), 607.97 (3.62), 559.67 (2.98), 528.70 (3.47), 500.64 (4.13), 402.14 (5.19). ^{1}H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: major isomer: 9.75 s (1H, 10-H), 9.71 s (1H, 5-H), 8.88 s (1H, 20-H), 8.15 d.d [1H, 3(1)-H, *J* = 17.9, 11.5 Hz], 6.40 d.d [1H, *trans*-3(2)-H,

J = 1.8, 19.6 Hz], 6.18 d.d [1H, *cis*-3(2)-H, J = 1.6, 11.5 Hz], 5.88 d and 5.08 d [1H each, 15(1)-H, J = 19 Hz], 4.4–4.6 m (2H, 18-H, 17-H), 3.84 s [3H, 15(3)-H], 3.68 s [3H, 17(4)-H], 2.78 s [3H, 13(1)-NCH_3], 3.4–4.0 m [14H, 12(1)-H, 1(1)-H, 7(1)-H, 13(1)-NCH_3, 8(1)-H], 2.1–2.7 m [4H, 17(1)-H, 17(2)-H], 1.75 m [6H, 18(1)-H, 8(2)-H]; minor isomer: 9.73 s (1H, 10-H), 9.68 s (1H, 5-H), 8.83 s (1H, 20-H), 8.13 d.d [1H, 3(1)-H, J = 17.7, 11.5 Hz], 6.40 d.d [1H, *trans*-3(2)-H, J = 1.8, 19.6 Hz], 6.18 d.d [1H, *cis*-3(2)-H, J = 1.6, 11.5 Hz], 5.73 d and 5.16 d [1H each, 15(1)-H, J = 19 Hz], 4.4–4.6 m (2H, 18-H, 17-H), 3.80 s [3H, 15(3)-H], 3.70 s [3H, 17(4)-H], 3.13 s [3H, 13(1)-NCH_3], 3.4–4.0 m [14H, 12(1)-H, 1(1)-H, 7(1)-H, 13(1)-NCH_3, 8(1)-H], 2.1–2.7 m [4H, 17(1)-H, 17(2)-H], 1.75 m [6H, 18(1)-H, 8(2)-H]. ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 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. Mass spectrum, m/z: 651 $[M]^+$, 620 $[M - OCH_3]^+$.

***N*,*N*-Diethyl-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (VII)** was synthesized from 80 mg of methylpheophorbide *a* (**I**); the product was isolated using CCl_4–acetone (12:1, by volume) as eluent. Yield 45 mg (50%). IR spectrum, ν, cm^{-1}: 1741 (C=O, ester), 1635 (C=O, amide I), 1612 (chlorin). ^1H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: major isomer: 9.73 s (1H, 10-H), 9.70 s (1H, 5-H), 8.87 s (1H, 20-H), 8.15 d.d [1H, 3(1)-H, J = 17.7, 11.5 Hz], 6.38 d.d [1H, *trans*-3(2)-H, J = 19.3, 1.5 Hz], 6.16 d.d [1H, *cis*-3(2)-H, J = 12.4, 1.5 Hz], 5.73 d and 5.10 d [1H each, 15(1)-H, J = 19.4], 4.49 m (1H, 18-H), 4.32 m (1H, 17-H), 3.87 s [3H, 15(3)-H], 3.64 s [3H, 17(4)-H], 3.56 s [3H, 12(1)-H], 3.53 s [3H, 2(1)-H], 3.36 s [3H, 7(1)-H], 3.72 m [2H, 8(1)-H], 3.18 m [2H, 13(2)-H], 2.98 m [3H, 13(3)-H], 2.0–2.7 m [4H, 17(1)-H, 17(2)-H], 1.77 d [3H, 18(1)-H, J = 7.1 Hz], 1.74 t [3H, 8(2)-H, J = 7.5 Hz]; minor isomer: 9.70 (1H, 10-H), 9.67 s (1H, 5-H), 8.83 s (1H, 20-H), 8.15 d.d [1H, 3(1)-H, J = 17.7, 11.5 Hz], 6.38 d.d [1H, *trans*-3(2)-H, J = 19.3, 1.5 Hz], 6.16 d.d [1H, *cis*-3(2)-H, J = 12.4, 1.5 Hz], 5.26 s (2H, 15(1)-H), 4.49 m (1H, 18-H), 4.32 m (1H, 17-H), 3.80 s [3H, 15(3)-H], 3.64 s [3H, 17(4)-H], 3.56 s [3H, 12(1)-H], 3.53 s [3H, 2(1)-H], 3.36 s [3H, 7(1)-H], 3.72 m [2H, 8(1)-H], 3.18 m [2H, 13(2)-CH], 2.98 m [3H, 13(3)-H], 2.0–2.7 m [4H, 17(1)-H, 17(2)-H], 1.65 m [6H, 18(1)-H, 8(2)-H]. Mass spectrum, m/z: 679 $[M]^+$, 648 $[M - OCH_3]^+$.

15(2),17(3)-Dimethoxychlorin e_6-13(1)-morpholide (VIII) was synthesized from 50.0 mg of methylpheophorbide *a* (**I**); the product was isolated using CCl_4–acetone (4:1, by volume) as eluent. Yield 24.0 mg (42%). IR spectrum, ν, cm^{-1}: 1742 (C=O, ester), 1636 (C=O, amide I), 1608 (chlorin). Electronic absorption spectrum, λ_{max}, nm (log ε): 663.84 (4.73), 608.73 (3.74), 557.05 (3.41), 528.52 (3.68), 500.54 (4.19), 402.17 (5.21). ^1H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: major isomer: 9.72 s (1H, 10-H), 9.67 s (1H, 5-H), 8.86 s (1H, 20-H), 8.11 d.d [1H, 3(1)-H, J = 17.0, 11.6 Hz], 6.38 d.d [1H, *trans*-3(2)-H, J = 17.9, 1.5 Hz], 6.16 d.d [1H, *cis*-3(2)-H, J = 11.6, 1.6 Hz], 5.81 d and 5.09 d [1H each, 15(1)-H, J = 19.0 Hz], 4.42–4.65 m (2H, 18-H, 17-H), 3.88 s [3H, 15(3)-H], 3.66 s [3H, 17(4)-H], 3.57 s [3H, 12(1)-H], 3.51 s [3H, 2(1)-H], 3.34 s [3H, 7(1)-H], 3.7–4.2 m [10H, 8(1)-H, 13(2)-H, 13(3)-H], 2.2–2.7 m [4H, 17(1)-H, 17(2)-H], 1.6–1.8 m [6H, 18(1)-H, 8(2)-H]; minor isomer: 9.69 s (1H, 10-H), 9.64 s (1H, 5-H), 8.81 s (1H, 20-H), 8.11 d.d [1H, 3(1)-H, J = 17.9, 11.5 Hz], 6.38 d.d [1H, *trans*-3(2)-H, J = 17.9, 1.5 Hz], 6.16 d.d [1H, *cis*-3(2)-H, J = 11.6, 1.6 Hz], 5.52 d and 5.25 d [1H each, 15(1)-H, J = 19.0 Hz], 4.42–4.65 m (2H, 18-H, 17-H), 3.83 s [3H, 15(3)-H], 3.62 s [3H, 17(4)-H], 3.54 s [3H, 12(1)-H], 3.50 s [3H, 1(1)-H], 3.33 s [3H, 7(1)-H], 3.7–4.2 m [10H, 8(1)-H, 13(2)-H, 13(3)-H], 2.2–2.7 m [4H, 17(1)-H, 17(2)-H], 1.6–1.8 m [6H, 18(1)-H, 8(2)-H]. ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 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. Mass spectrum, m/z: 693 $[M]^+$, 662 $[M - OCH_3]^+$.

By-products in the reaction of methylpheophorbide *a* (I) with 2-aminoethanol. After isolation of amide **V**, the column was eluted with chloroform–

methanol (9:1) to isolate fractions containing diamide **X** and triamide **XII**. Reprecipitation from chloroform with pentane gave 8 mg of **X** and 18 mg of **XII**. Compounds **X** and **XII** were also characterized as the corresponding acetates.

Compound **X**. IR spectrum, ν, cm^{-1}: 1740 (C=O, ester), 1645 (C=O, amide I), 1610 (chlorin), 1526 (δNH, amide II).

Compound **XII**. IR spectrum, ν, cm^{-1}: 3100 (NH, amide), 1650 (C=O, amide I), 1610 (chlorin), 1550 (δNH, amide II). Electronic absorption spectrum, λ_{max}, nm (logε): 663.69 (4.30), 609.64 (3.34), 557.46 (2.99), 529.98 (3.32), 500.93 (4.78), 402.99 (4.82).

***N*,*N*′-Bis(2-hydroxyethyl)-15(2)-methoxychlorin e_6-13(1),17(3)-diamide (X).** *a*. A solution of 40 mg of amide **V** in 0.5 ml of 2-aminoethanol was held for 8 h at room temperature in the dark. The mixture was applied to a column charged with silica gel (the column was packed by the "moist" technique with carbon tetrachloride), and the column was eluted first with carbon tetrachloride and then with chloroform–methanol (9:1). The fraction containing the target product was evaporated, and the residue was reprecipitated from chloroform with pentane. Yield 26 mg (62%).

b. 2-Aminoethanol, 0.5 ml, was added to a solution of 60 mg of methylpheophorbide *a* (**I**) in 10 ml of chloroform, the mixture was stirred for 2 h at room temperature in the dark, the solvent was distilled off under reduced pressure, and the liquid residue was left to stand for 8 h at room temperature in the dark. The mixture was then treated as described above in *a*. Yield 39 mg (57%).

***N*,*N*′,*N*″-Tris(2-hydroxyethyl)chlorin e_6-13(1),-15(2),17(3)-triamide (XII).** *a*. A solution of 42 mg of amide **V** in 0.5 ml of 2-aminoethanol was held for 60 h at room temperature in the dark. The mixture was then treated as described above for compound **X**. Yield 23 mg (50%).

b. 2-Aminoethanol, 0.5 ml, was added to a solution of 65 mg of methylpheophorbide *a* (**I**) in 10 ml of chloroform, the mixture was stirred for 2 h at room temperature in the dark, the solvent was distilled off under reduced pressure, and the liquid residue was left to stand for 60 h at room temperature in the dark. The mixture was then treated as described above in *a*. Yield 34 mg (44%).

Acetylation of amides containing 2-hydroxyethylamino groups. Initial amide was dissolved in a mixture of 0.3 ml of acetic anhydride and 0.5 ml of pyridine. The mixture was held for 1 h at room temperature in the dark, diluted with 100 ml of chloroform, washed in succession with 5% hydrochloric acid (3×50 ml) and water until neutral washings, dried over anhydrous sodium sulfate, and evaporated. The residue was subjected to chromatography on silica gel, followed by reprecipitation from chloroform with pentane.

***N*-(2-Acetoxyethyl)-15(2),17(3)-dimethoxychlorin e_6-13(1)-amide (IX)** was synthesized from 42 mg of amide **V**; the product was isolated using CCl_4–acetone (20:1) as eluent. Yield 43 mg (96%). Electronic absorption spectrum, λ_{max}, nm (logε): 663.34 (4.06), 607.98 (3.32), 558.12 (3.67), 528.62 (3.29), 500.59 (3.61), 402.21 (4.55). ^{1}H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: 9.69 s (1H, 10-H), 9.63 s (1H, 5-H), 8.79 s (1H, 20-H), 8.08 d.d [1H, 3(1)-H, *J* = 17.8, 11.5 Hz], 6.35 d.d [1H, *trans*-3(2)-H, *J* = 17.9, 1.4 Hz], 6.14 d.d [1H, *cis*-3(2)-H, *J* = 1.6, 11.5 Hz], 6.67 br.t [1H, 13(1)-NH, *J* = 5.7 Hz], 5.52 d and 5.26 d [1H, 15(1)-H, *J* = 18.8 Hz], 3.85–4.50 m [6H, 18-H, 17-H, 13(2)-H, 13(3)-H], 3.82 s [3H, 15(3)-H], 3.60 s [3H, 17(4)-CH_3], 3.56 s [3H, 12(1)-H], 3.48 s [3H, 2(1)-H], 3.31 s [3H, 7(1)-H], 3.75–3.85 m [2H, 8(1)-H], 2.11 s [3H, 13(5)-H], 2.1–2.6 m [4H, 17(1)-H, 17(2)-H], 1.72 t [3H, 8(2)-H, *J* = 7.8 Hz], 1.71 d [3H, 18(1)-H, *J* = 7.2 Hz], –1.55 br.s (1H, *I*-NH), –1.75 br.s (1H, *III*-NH). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 11.35, 11.90, 12.15, 17.69, 19.71, 20.89, 23.03, 29.72, 31.13, 37.90, 39.07, 51.59, 52.15, 49.30, 53.09, 63.35, 93.73, 98.85, 101.51, 121.73, 127.78, 129.48, 129.88, 130.33, 134.72, 135.00, 135.00, 135.00, 136.10, 139.08, 145.00, 171.23, 168.99, 169.62, 173.50, 173.97. Mass spectrum: *m*/*z* 709 $[M]^+$.

***N*,*N*′-Bis(2-acetoxyethyl)-15(2)-methoxychlorin e_6-13(1),17(3)-diamide (XI)** was synthesized from 18 mg of diamide **X**; the product was isolated using CCl_4–acetone (12:1) as eluent. Yield 14 mg (69%). Electronic absorption spectrum, λ_{max}, nm (logε): 662.97 (4.77), 607.46 (3.77), 559.53 (3.312), 528.45 (3.66), 500.55 (4.23), 402.20 (5.29). ^{1}H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: 9.70 s (1H, 10-H), 9.62, 9.68 s (1H, 5-H), 8.78 s (1H, 20-H), 8.08 d.d [1H, 3(1)-H, *J* = 18.5, 11.5 Hz], 6.36 d.d [1H, *trans*-3(2)-H, *J* = 17.8, 1.4 Hz], 6.15 d.d [1H, *cis*-3(2)-H, *J* = 11.6, 1.0 Hz], 6.76 br.t [1H, 13(1)-NH, *J* = 5.2 Hz], 5.04 br.t [1H, 17(3)-NH, *J* = 5.6 Hz], 5.42 d and 5.37 d [1H each, 15(1)-H, *J* = 19.0 Hz], 4.07 m (1H, 18-H), 3.97 m (1H, 17-H), 3.5–4.2 m [2H, 13(2)-H], 4.5 t [2H, 13(3)-H, *J* = 5.4 Hz], 3.05 m [2H, 17(5)-H], 3.83 s [3H, 15(3)-H], 3.56 s [3H, 12(1)-H], 3.48 s [3H, 2(1)-H], 3.31 s [3H, 7(1)-H], 3.97 m [2H, 8(1)-H],

1.9–2.4 m [4H, 17(1)-H, 17(2)-H], 1.7–1.8 m [6H, 8(2)-H, 18(1)-H], 2.12 s [3H, 13(5)-H], 1.75 s [3H, 17(7)-H], –1.55 br.s (1H, *I*-NH), –1.72 br.s (1H, *III*-NH). ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 11.34, 11.91, 12.16, 17.70, 19.70, 20.60, 20.91, 23.31, 29.71, 30.73, 38.04, 38.20, 39.61, 49.54, 52.22, 52.95, 62.91, 63.31, 93.42, 98.81, 101.61, 102.37, 121.83, 127.72, 129.41, 129.72, 130.49, 134.80, 134.80, 135.12, 135.12, 136.13, 139.15, 144.97, 149.04, 154.49, 169.59, 166.08, 170.72, 171.04, 172.39, 172.39, 174.30. Mass spectrum: *m*/*z* 780 $[M]^+$.

***N*,*N*′,*N*″-Tris(2-acetoxyethyl)chlorin e_6-13(1),-15(2),17(3)-triamide (XIII)** was synthesized from 15 mg of triamide **XII**; the product was isolated using CCl_4–acetone (10:1) as eluent. Yield 11 mg (63%). IR spectrum, ν, cm^{-1}: 1720 (C=O, ester), 1650 (C=O, amide I), 1610 (chlorin), 1545 (δNH, amide II), 1250 (ester), 1060 (δC–O–C, ester). Electronic absorption spectrum, λ_{max}, nm (log ε): 663.69 (4.30), 609.64 (3.34), 557.46 (2.99), 529.98 (3.32), 500.93 (4.78), 402.99 (4.82). ^{1}H NMR spectrum ($CDCl_3$, 300 MHz), δ, ppm: 9.71 s (1H, 10-H), 9.63 s (1H, 5-H), 8.81 s (1H, 20-H), 8.09 d.d [1H, 3(1)-H, *J* = 17.7, 11.6 Hz], 6.36 d [1H, *trans*-3(2)-H, *J* = 17.9 Hz], 6.16 d [1H, *cis*-3(2)-H, *J* = 11.6 Hz], 6.88 br.t [1H, 13(1)-NH, *J* = 5.7 Hz], 5.42 br.t [1H, 17(3)-NH, *J* = 5.7 Hz], 7.48 br.t [1H, 15(2)-NH, *J* = 5.7 Hz], 5.29 m [2H, 15(1)-H], 4.47 br.q (2H, 18-H, *J* = 7.0 Hz), 4.40 m (1H, 17-H), 3.40 m [2H, 13(2)-H], 4.11–4.02 m [4H, 13(3)-H, 15(4)-H], 4.52 br.t [2H, 17(4)-H, *J* = 6 Hz], 3.58 s [3H, 12(1)-H], 3.49 s [3H, 2(1)-H], 3.31 s [3H, 7(1)-H], 3.80–3.78 m [6H, 17(5)-H, 15(3)-H, 8(1)-H], 2.30–2.27 m [2H, 17(2)-H], 1.9–1.7 m [2H, 17(1)-H], 1.73–1.70 m [6H, 8(2)-H, 18(1)-H], 2.15 s [3H, 13(5)-H], 1.90 s [3H, 17(7)-H], 1.52 s [3H, 15(6)-H]. ^{13}C NMR spectrum ($CDCl_3$), δ_C, ppm: 11.72, 12.36, 12.60, 18.14, 20.07, 20.78, 21.14, 21.40, 23.65, 30.12, 30.61, 38.80, 39.07, 39.96, 40.35, 49.54, 53.25, 63.17, 63.31, 63.74, 94.06, 99.31, 101.81, 103.21, 122.25, 128.70, 129.78, 130.33, 130.71, 135.08, 135.20, 135.33, 135.60, 136.60, 139.31, 145.00, 168.94, 170.22, 171.24, 171.34, 171.75, 173.03, 173.61. Mass spectrum: *m*/*z* 851 $[M]^+$.

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