ISSN 1070-4280, Russian Journal of Organic Chemistry, 2009, Vol. 45, No. 3, pp. 452–459. © Pleiades Publishing, Ltd., 2009.
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Transformations of the Extra Ring in Pheophorbide *a* Methyl Ester in the Reaction with *N*,*N*,*N*′,*N*′-Tetramethylmethanediamine

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Received June 13, 2008

Abstract—The reaction of *N*,*N*,*N*′,*N*′-tetramethylmethanediamine with pheophorbide *a* methyl ester gave the corresponding 13″-dimethylaminomethyl derivative and 13-*N*,*N*-dimethylamide derivative of chlorin e_6 having a methyl acrylate moiety in the 15-position. Conditions were found for the synthesis of the latter both directly from pheophorbide *a* methyl ester and from its aminomethylation product. Probable mechanisms of the examined reactions were proposed.

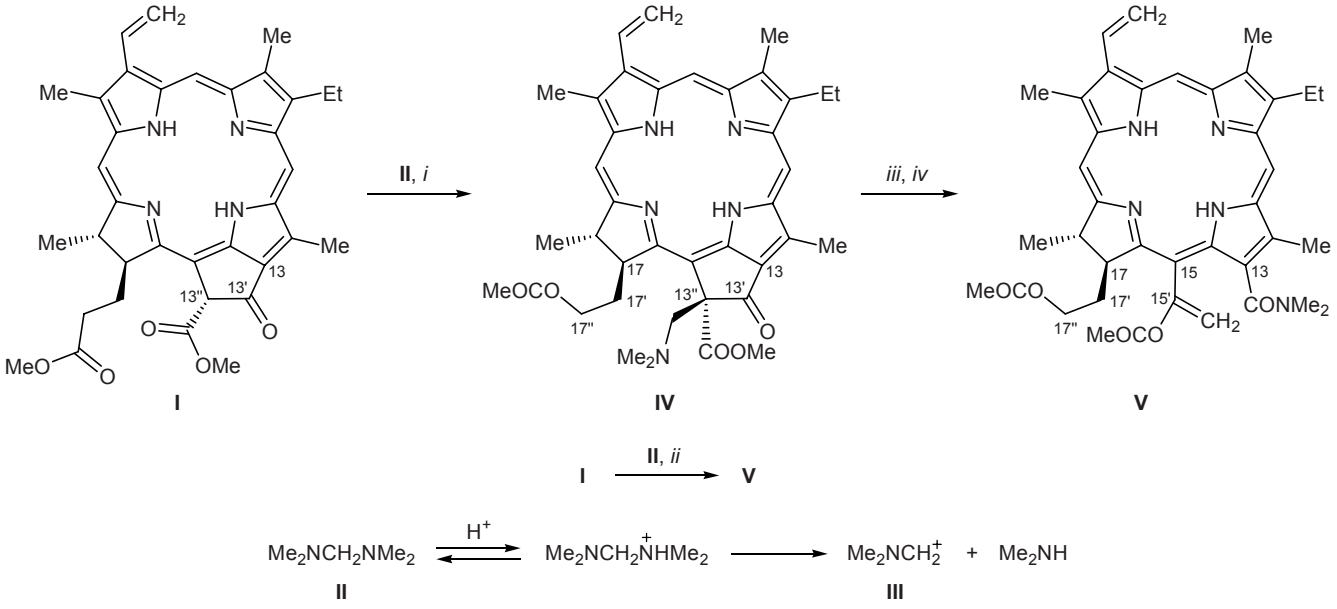
DOI: 10.1134/S1070428009030191

Development of procedures for chemical modification of chlorophyll *a* derivatives, such as pheophorbide *a* methyl ester (**I**), attracts strong interest from the viewpoint of design of antitumor agents [1, 2]. The extra ring in molecule **I** and its analogs is a reaction center capable of undergoing various transformations, many of which are useful for the synthesis of chlorin derivatives [3–7]. It is known [8] that *N*,*N*,*N*′,*N*′-tetramethylmethanediamine (**II**) is a convenient reagent for generation of dimethylaminomethyl cation (**III**) (Scheme 1). Analogous cation is formed in Mannich reactions. Reactions of electrophilic species **III** with carbonyl compounds lead to the formation of the corresponding dimethylaminomethyl derivatives. Therefore, reaction of diamine **II** with compound **I** may be promising from the viewpoint of synthesis of new chlorins.

In the present work we examined reactions of *N*,*N*,*N*′,*N*′-tetramethylmethanediamine (**II**) with pheophorbide *a* methyl ester (**I**) under different conditions (Scheme 1). When the reaction was carried out at 10–12°C in a mixture of tetrahydrofuran with acetic acid, aminomethylation at the extra ring in the α-position with respect to the oxo group gave 13″-dimethylaminomethyl derivative **IV** whose structure was confirmed by the ^{1}H NMR and IR data. Unlike initial pheophorbide *a* methyl ester (**I**), the ^{1}H NMR spectrum of **IV** lacked singlet corresponding to the 13″-H proton, while signals typical of dimethylaminomethyl group appeared (Fig. 1). In the IR spectrum of **IV** we observed absorption bands due to stretching vibrations of methylene C–H bonds in the dimethylaminomethyl substituent. Protons of the NCH_2 group resonated in the ^{1}H NMR spectrum as an *AB* multiplet (Fig. 1), and protons in the methyl groups on the nitrogen atom gave rise to a singlet at δ 1.84 ppm.

The mass spectrum of compound **IV** did not provide unambiguous proofs for its structure. Although the mass spectrum contained the molecular ion peak corresponding to structure **IV**, the main fragment ions conformed to isomeric molecular ion **V**. Most probably, heating of a sample of **IV** during GC–MS analysis promoted its isomerization into compound **V** (see below), so that just the latter underwent ionization and subsequent fragmentation. Aminomethylation of pheophorbide *a* methyl ester (**I**) was stereoselective, and the product was exclusively (13″*R*)-diastereoisomer. The configuration of $C^{13''}$ in molecule **IV** was determined on the basis of the ROESY data: cross peaks corresponding to interaction between protons in the dimethylaminomethyl group on $C^{13''}$ and those in the propionate moiety on C^{17} were more intense than cross

Scheme 1.

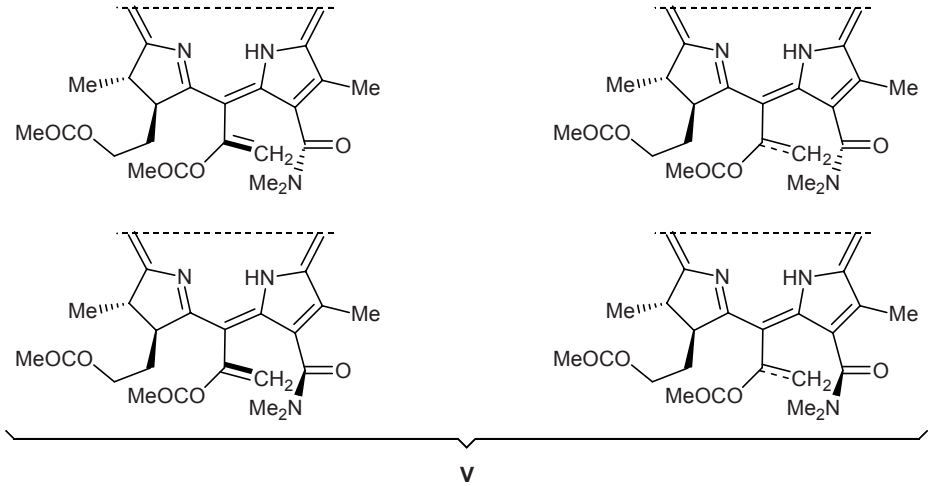


i: THF–AcOH, 10–12°C, 24 h; *ii*: THF, reflux, 8 h; *iii*: THF–AcOH, 48 h, 20°C; *iv*: THF, reflux.

peaks originating from interaction between protons in the 17-propionate group and 13″-methoxycarbonyl group. These findings indicated that both 13″-dimethylaminomethyl and 17-propionate group are located at the same side of the macroring.

The observed stereoselectivity may be rationalized in terms of specificity of the substrate structure. Presumably, the enol tautomer of **I** is not completely planar, and the 13″-COOMe group is slightly displaced toward transoid orientation with respect to the 17-CH_2CH_2COOMe group as a result of mutual repulsion of these substituents. Therefore, electrophilic attack on the double bond in the enol form occurs at the sterically more accessible side which is opposite to the 13″-COOMe group, and only one diastereoisomer with the 13″-dimethylaminomethyl group and 17-propionate fragment located at the same side of the macroring is formed.

13″-Dimethylaminomethyl derivative **IV** underwent isomerization to chlorin e_6 derivative **V** on prolonged keeping in a mixture of THF with acetic acid at room temperature (Schemes 1–3). The structure of **V** was confirmed by the NMR, IR, and mass spectra. The IR spectrum of **V** lacked absorption band typical of the $C^{13'}$=O carbonyl in structure **IV**, which indicated cleavage of the extra ring. Instead, amide I band appeared due to 13-$CONMe_2$ group. The mass spectrum of **V** contained peaks from the singly and doubly



V

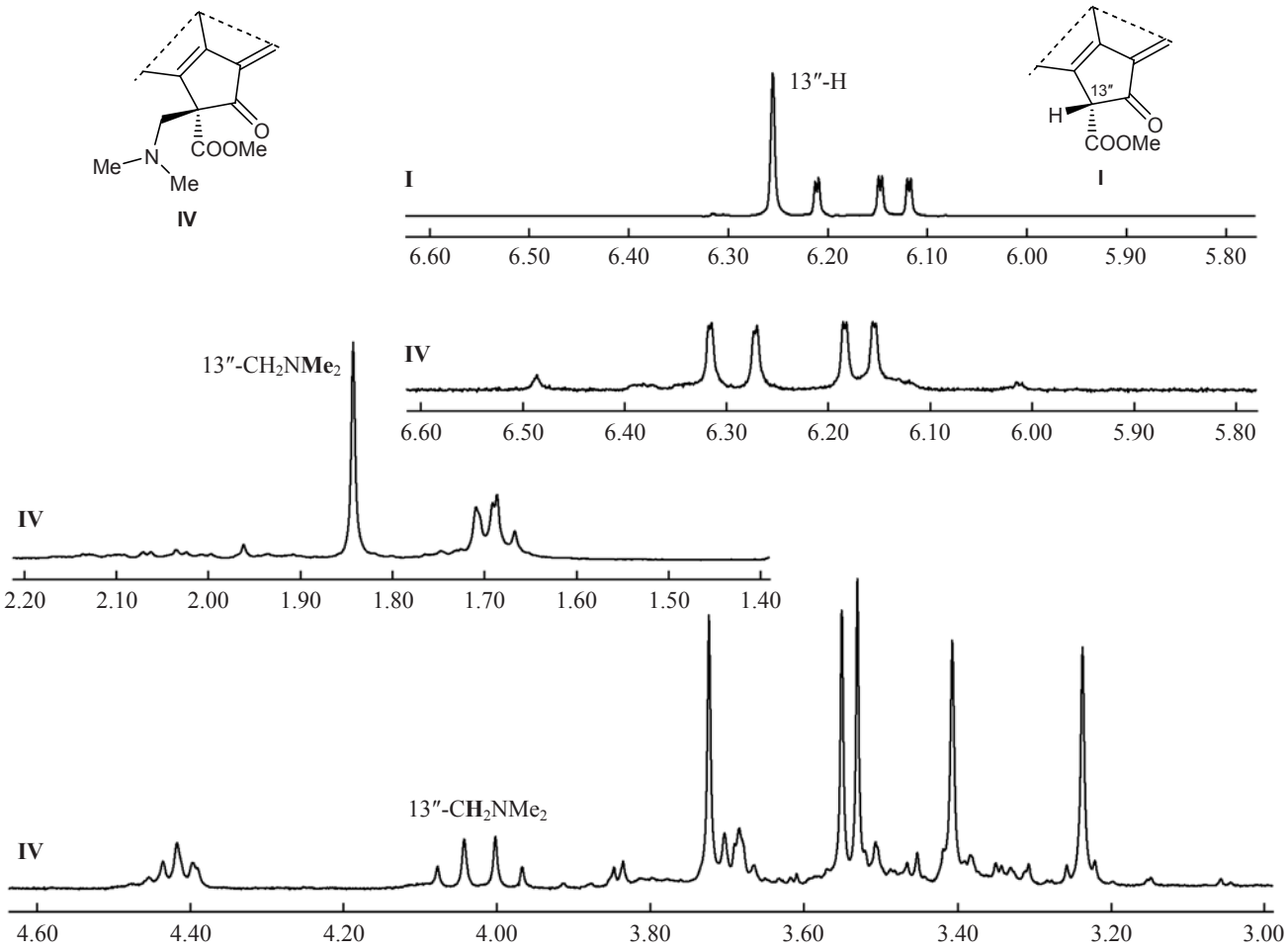


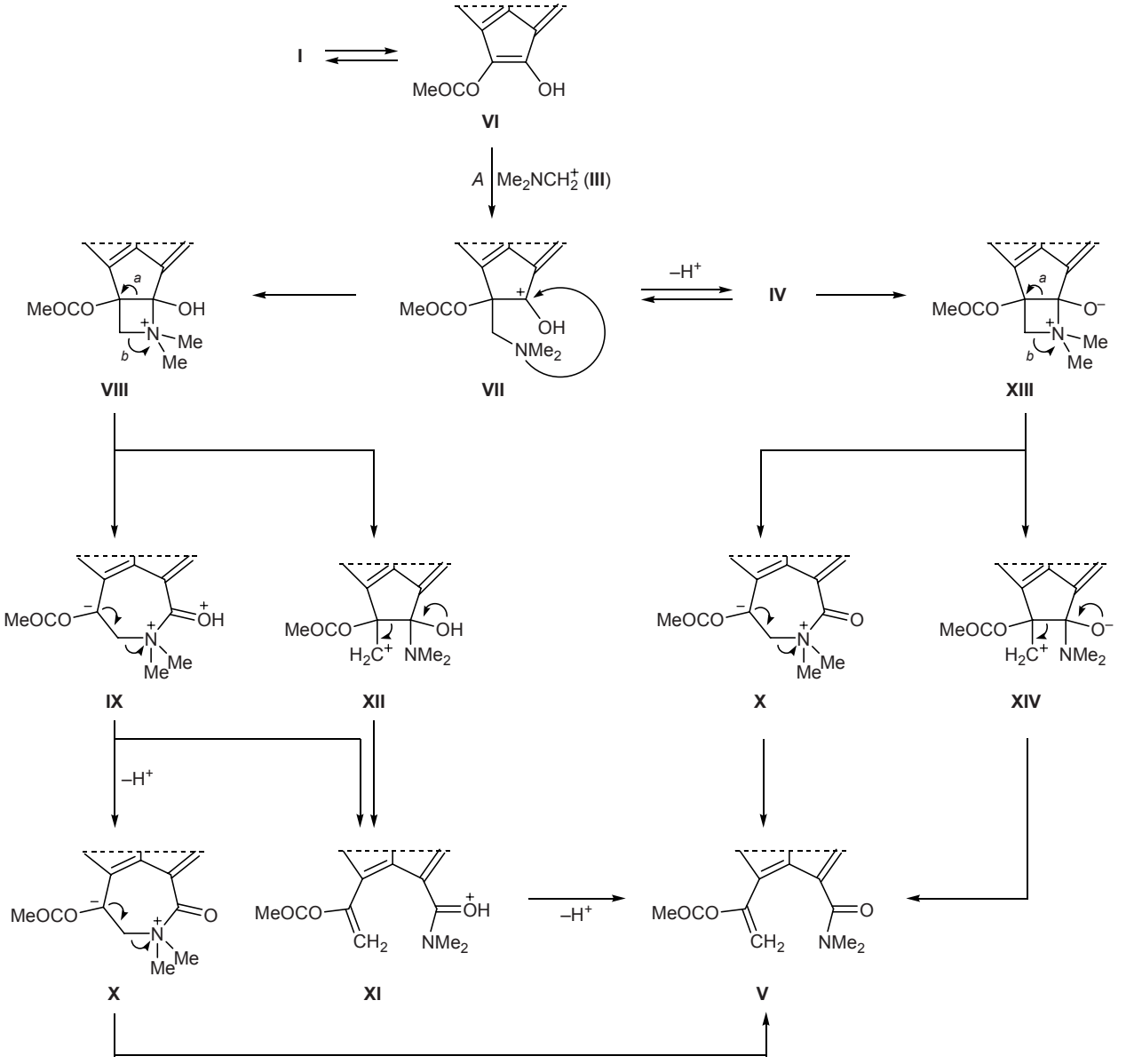
Fig. 1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$; δ, ppm) of product **IV** formed in the reaction of *N*,*N*,*N*′,*N*′-tetramethylmethanediamine (**II**) with pheophorbide *a* methyl ester (**I**).

charged molecular ions and a number fragment ion peaks. Unlike many chlorophyll *a* derivatives which are characterized by elimination of the 17-propionate moiety from the molecular ion, the main fragmentation pathway of the molecular ion of **V** involved elimination of the 13-carbamoyl group. Presumably, elimination of the carboxamide moiety reduces steric strain in the molecule, which makes this fragmentation pathway more favorable.

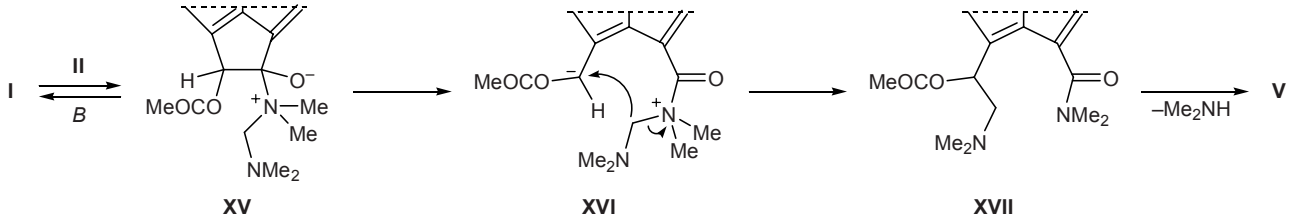
Compound **V** displayed in the ^{1}H NMR spectrum doublets from the vinyl protons in the 15-C=CH_2 fragment and singlets from the methyl protons in the 13-$CONMe_2$ group (Fig. 2). The signals were assigned by comparing with the spectrum of chlorin e_6 15,17-dimethyl ester 13-*N*,*N*-dimethylamide described by us previously [6, 7] (Scheme 1).

NMR study on compound **V** showed that it exists as a mixture of four isomers. Its ^{1}H NMR spectrum may be interpreted as a superposition of spectra of four chlorin derivatives characterized by the same number and multiplicities of signals but differing in their positions. The largest differences in the chemical shifts were observed for vinyl protons in the acrylic acid ester fragment on C^{15} and *N*-methyl protons in the amide group on C^{13} (Fig. 2; see Experimental). We believe that compound **V** gives rise to four atropisomers differing by mutual arrangement of the chlorin macroring, 13-$CONMe_2$, and 15-C(=CH_2)COOMe planes (Scheme 1) due to restricted rotation about the C^{13}–$C^{13'}$ and C^{15}–$C^{15'}$ single bonds. Analogous pattern was observed while studying chlorin e_6 derivatives with *N*,*N*-disubstituted carboxamide group in the 13-position, e.g., chlorin e_6 15,17-dimethyl ester 13-*N*,*N*-dimethylamide (**XVIII**). Such compounds were found to exist as two isomers with different orientations of the 13-carboxamide group with respect to the macroring [6, 7]. The number of atropisomers can be determined by analysis of signals from protons in the *meso* positions (Fig. 3). Only one atropisomer

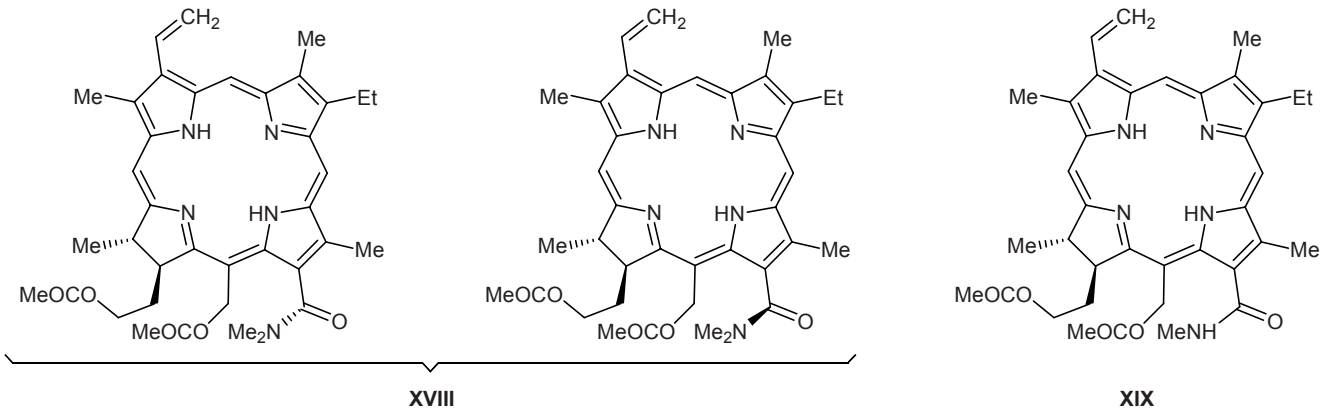
Scheme 2.



Scheme 3.



was found for *N*-methyl derivative **XIX**, and its ^{1}H NMR spectrum contained only one set of signals from the *meso* protons. Two atropisomers of chlorin e_6 **XVIII** give rise to two sets of signals from the *meso* protons. Likewise, four sets of signals are observed in the ^{1}H NMR spectrum of **V** due to the presence of four atropisomers. The same applies to other proton signals in the spectra of chlorine e_6 13-amides; however, signals of particular atropisomers are not always well resolved (Fig. 2).



XVIII XIX

Probable mechanisms of formation of compound **V** are shown in Schemes 2 (path *A*) and 3 (path *B*). The first of these includes formation of aminomethylation product **IV** and its subsequent isomerization. According to the second path, nucleophilic attack on the $C^{13'}$ carbonyl carbon atom is followed by cleavage of the

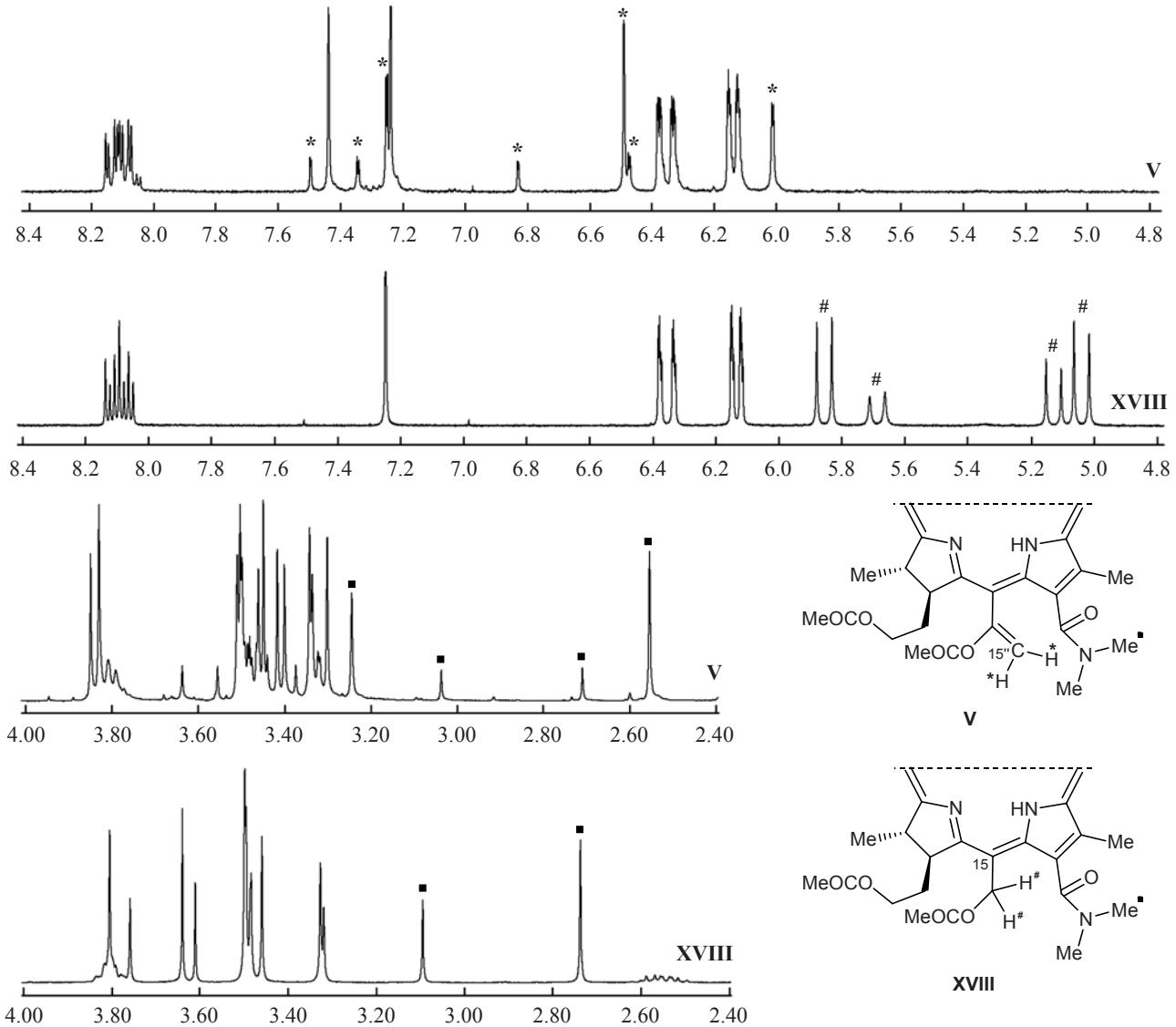


Fig. 2. ^{1}H NMR spectra (400 MHz, $CDCl_3$; δ, ppm) of chlorin e_6 derivatives **V** and **XVIII** (for comments, see text).

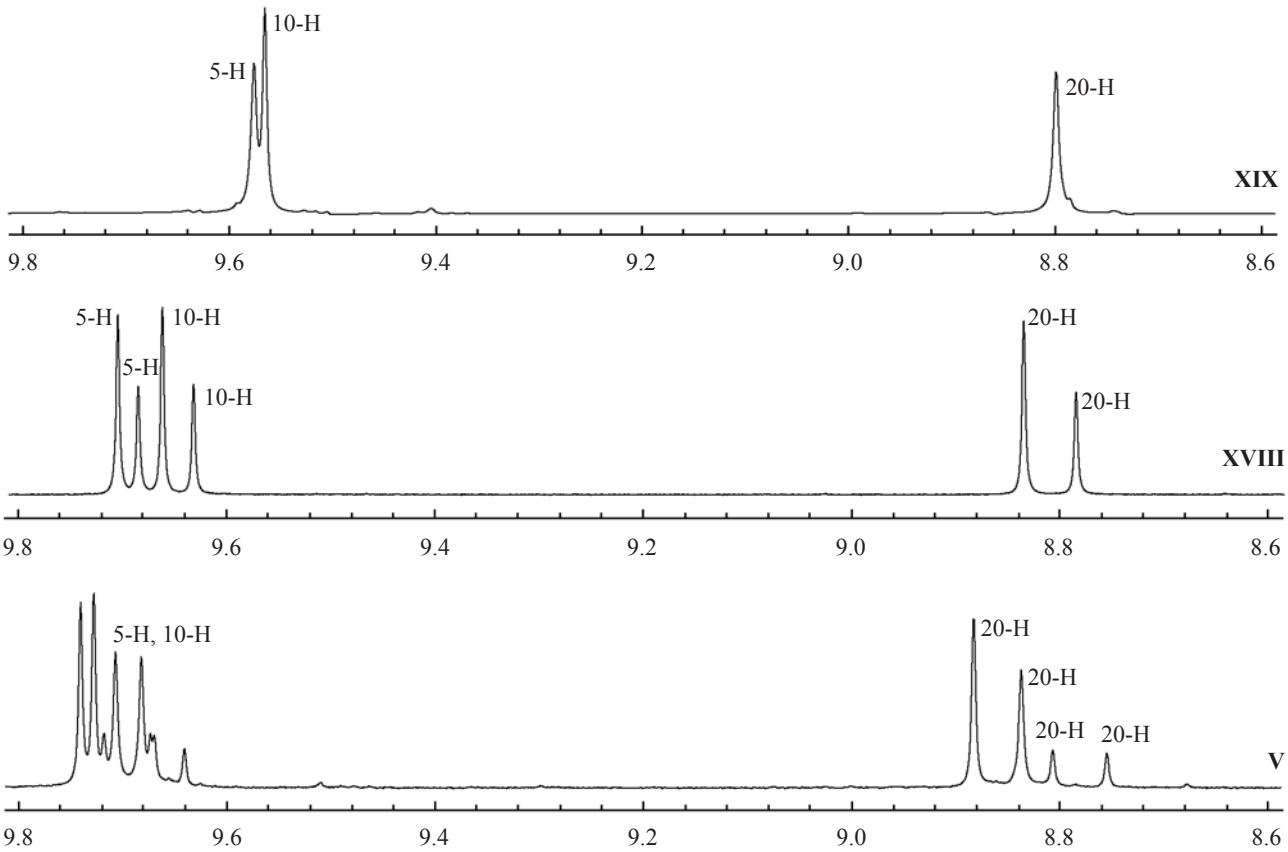


Fig. 3. ^{1}H NMR spectra (400 MHz, $CDCl_3$; δ, ppm; region corresponding to resonance of *meso*-protons) of chlorin e_6 derivatives **V**, **XVIII**, and **XIX**.

extra ring, migration of dimethylaminomethyl cation (**III**) to the $C^{15'}$ atom, and elimination of dimethylamine molecule with formation of double C=C bond.

The probability of path *A* is confirmed by the formation of aminomethylation product **IV** and its chemical properties. As shown above, compound **IV** can be obtained by treatment of pheophorbide *a* methyl ester (**I**) with diamine **II** at 10–12°C in a mixture of THF with acetic acid. The presence of acetic acid in the reaction mixture ensures generation of cation **III**, while reduced temperature prevents isomerization of **IV**. Compound **IV** is converted into chlorin **V** on heating in boiling THF in the absence of any other reagents. Under these conditions, the complete conversion of **IV** is attained only on prolonged heating (10 h), and the yield of isomerization product **V** is low (~20%). The occurrence of isomerization of **IV** in boiling THF, as well as on heating in mass spectrometer (see above), confirms possible intermediacy of this compound in the reaction of diamine **II** with pheophorbide *a* methyl ester (**I**) (Scheme 1). The presence in the reaction mixture of a weak acid favors the transformation of **IV** into **V**. The isomerization of **IV** may also be performed at room temperature in a 1 : 1 mixture of THF with acetic acid (reaction time 48 h). In this case, the yield of **V** increases to 45%.

The presence of an acid is likely to facilitate the isomerization of **IV** into **V** via protonation of the $C^{13'}$=O carbonyl group, which favors intramolecular nucleophilic attack by the dimethylamino group. Presumably, intermediate **VII** is converted into chlorin **V**, which is also consistent with the proposed mechanism. On the other hand, protonation of the $C^{13'}$=O group is not a necessary condition, for chlorin **V** can be formed both from cation **VII** and directly from compound **IV** (Scheme 2).

The probability for path *B* is supported by the fact that compound **V** can be obtained directly from pheophorbide *a* methyl ester (**I**) by heating with diamine **II** in THF in the absence of acetic acid. In this case, no aminomethylation product **IV** was detected. Presumably, in the absence of acid, i.e., under conditions when generation of cation **III** is hindered, no aminomethylation of compound **I** at position 13″ occurs (Scheme 3).

Instead, attack by the dimethylamino group in **II** on the $C^{13'}$ carbonyl carbon atom leads to opening of the extra ring, followed by migration of relatively stable dimethylaminomethyl cation **III** to the $C^{15'}$ carbon atom. Heterolytic dissociation of the C–N bond in zwitterionic intermediate **XVI** is quite feasible under severe conditions. Analogous cleavage of the extra ring in compound **I** and its analogs by the action of primary or secondary amines was reported in [5] with the difference that the reaction involved migration of proton to $C^{15'}$. Elimination of diethylamine molecule from intermediate **XVII** yields compound **V**. This process is favored by elevated temperature and stabilization of the newly formed double bond in **V** via conjugation with π-electrons in the ester group. Thus path *B* cannot be ruled out completely, especially in the reaction of pheophorbide *a* methyl ester (**I**) with diamine **II** in boiling THF.

To conclude, the reaction of pheophorbide *a* methyl ester (**I**) with *N*,*N*,*N*′,*N*′-tetramethylmethanediamine (**II**), depending on the conditions, could give previously unknown 13″-(dimethylaminomethyl)-substituted pheophorbide *a* methyl ester (**IV**) or chlorin e_6 derivative **V** containing a methyl acrylate fragment at the 15-position and dimethylcarbamoyl group on C^{13}. Compound **V** can be obtained both directly from pheophorbide *a* methyl ester (**I**) and from its aminomethylation product **IV**.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Bruker AMX-400 instrument (400 MHz) from solutions in $CDCl_3$. The IR spectra were measured in KBr on a Specord M-80 spectrometer. The mass spectra (electron impact, 70 eV) were obtained on a Thermo DSQ mass spectrometer with direct sample admission into the ion source. Silica gel (40–100 mesh; Lachema) was used for preparative column chromatography. Pheophorbide *a* methyl ester (**I**) was isolated from blue–green algae Spirulina [9]. The spectral parameters of **I** were consistent with those reported previously [7].

13″-(*N*,*N*-Dimethylaminomethyl)pheophorbide *a* methyl ester (IV). *N*,*N*,*N*′,*N*′-Tetramethylmethanediamine (**II**), 1.5 ml (11.70 mmol), was added to a solution of 100 mg (0.16 mmol) of pheophorbide *a* methyl ester (**I**) in a mixture of 10 ml of THF and 10 ml of acetic acid. The mixture was kept for 24 h at 10–12°C, diluted with chloroform, washed with water, dried over Na_2SO_4, and evaporated to dryness under reduced pressure at 25–30°C. The residue was reprecipitated from chloroform with pentane. Yield 82 mg (75%), dark blue crystals. IR spectrum, ν, cm^{-1}: 2876 (C–H); 1742 (C=O, ester); 1706 (C=O, ketone); 1618 ("chlorin band"); 1164, 1044 (C–O–C). ^{1}H NMR spectrum, δ, ppm: –1.62 br.s and 0.24 br.s (1H each, NH), 1.69 t (3H, $C^{8''}H_3$, J = 7.6 Hz), 1.70 d (3H, $C^{18'}H_3$, J = 7.2 Hz), 1.84 s (6H, NMe_2), 1.87–2.21 m (2H, $C^{17''}H_2$), 2.22–2.79 m (2H, $C^{17'}H_2$), 3.24 s (3H, 7-CH_3), 3.40 s (3H, 2-CH_3), 3.53 s (3H, 12-CH_3), 3.55 s (3H, 17″-$COOCH_3$), 3.72 s (3H, 13″-CO_2CH_3), 3.66–3.74 m (2H, 8-CH_2), 3.99 d and 4.06 d (1H each, CH_2N, J = 14.0 Hz), 4.39–4.42 m (1H, 17-H), 4.43 q (1H, 18-H, J = 7.2 Hz), 6.17 d.d (1H, 3″-H_{cis}, J = 12.0, 1.2 Hz), 6.53 d.d (1H, 3″-H_{trans}, J = 17.6, 1.2 Hz), 8.00 d.d (1H, 3′-H, J = 17.6, 12.0 Hz), 8.57 s (1H, 20-H), 9.39 s (1H, 5-H), 9.55 s (1H, 10-H).

15′-Methylidenechlorin e_6 17‴,15″-dimethyl ester 13′-*N*,*N*-dimethylamide (V). *a*. Diamine **II**, 1.5 ml (11.70 mmol), was added to a solution of 142 mg (0.23 mmol) of pheophorbide *a* methyl ester (**I**) in 15 ml of THF. The mixture was heated for 8 h under reflux, diluted with chloroform, washed with water, dried over Na_2SO_4, and evaporated to dryness under reduced pressure at 40–50°C. The residue was subjected to column chromatography on silica gel using carbon tetrachloride–acetone (50 : 1 to 1 : 1) as eluent; the product was additionally purified by reprecipitation from chloroform with pentane. Yield 60 mg (40%), dark green crystals. IR spectrum, ν, cm^{-1}: 1740, 1730 (C=O, ester); 1644 (C=O, amide I); 1606 ("chlorin band"); 1164, 1066 (C–O–C). ^{1}H NMR spectrum, δ, ppm: isomer **1** (47%, the isomer ratio was determined from the intensities of the 20-H signals): –2.09 br.s and –1.90 br.s (1H each, NH), 1.72 t (3H, $C^{8''}H_3$, J = 8.0 Hz), 1.66 d (3H, 18-CH_3, J = 6.8 Hz), 1.98–1.78 m (2H, $C^{17''}H_2$), 2.40–2.10 m (2H, $C^{17'}H_2$), 2.55 s and 3.45 s (3H each, NCH_3), 3.30 s (3H, 7-CH_3), 3.42 s (3H, 2-CH_3), 3.51 s (3H, 12-CH_3), 3.50 s (3H, 17″-$COOCH_3$), 3.83 s (3H, 15′-$COOCH_3$), 3.76–3.84 m (2H, $C^{8'}H_2$), 4.11–4.08 m (1H, 17-H), 4.46 q (1H, 18-H, J = 7.2 Hz), 6.14 d.d (1H, 3″-H_{cis}, J = 11.0, 1.4 Hz), 6.53 d.d (1H, 3′-H_{trans}, J = 17.6, 1.4 Hz), 7.44 d and 6.49 d (1H each, J 0.5 Hz) ($C^{15'}$=CH_2), 8.11 d.d (1H, 3′-H, J = 17.6, 11.6 Hz), 8.88 s (1H, 20-H), 9.73 s (1H, 5-H), 9.74 s (1H, 10-H); isomer **2** (37%): –1.92 br.s and –1.90 br.s (1H each, NH), 1.75 t (3H, $C^{8''}H_3$, J = 7.6 Hz), 1.72 d (3H, 18-CH_3, J = 8.0 Hz), 1.98–1.78 m (2H, $C^{17''}H_2$), 2.40–2.10 m (2H, $C^{17'}H_2$), 3.24 s and 3.46 s (3H each, NCH_3), 3.33 s (3H, 7-CH_3), 3.40 s (3H, 2-CH_3), 3.49 s

(3H, 12-CH_3), 3.50 s (3H, 17″-$COOCH_3$), 3.85 s (3H, 15′-$COOCH_3$), 3.76–3.84 m (2H, $C^{8'}H_2$), 4.11–4.08 m (1H, 17-H), 4.47 q (1H, 18-H, J = 7.2 Hz), 6.13 d.d (1H, 3″-H_{cis}, J = 12.0, 1.6 Hz), 6.53 d.d (1H, 3″-H_{trans}, J = 18.0, 1.6 Hz), 7.25 d and 6.01 d (1H each, $C^{15'}$=CH_2, J = 2.0 Hz), 8.11 d.d (1H, 3′-H, J = 18.0, 11.6 Hz), 8.84 s (1H, 20-H), 9.68 s (1H, 5-H), 9.71 s (1H, 10-H); isomer **3** (9%): –1.73 br.s and –1.64 br.s (1H each, NH), 1.70–1.74 m (3H, $C^{8''}H_3$), 1.62 d (3H, 18-CH_3, J = 7.2 Hz), 1.98–1.78 m (2H, $C^{17''}H_2$), 2.40–2.10 m (2H, $C^{17'}H_2$), 2.71 s and 3.46 s (3H each, NCH_3), 3.32 s (3H, 7-CH_3), 3.37 s (3H, 2-CH_3), 3.48 s (3H, 12-CH_3), 3.49 s (3H, 17″-$COOCH_3$), 3.55 (3H, 15′-$COOCH_3$), 3.76–3.84 m (2H, $C^{8'}H_2$), 4.67 br.d (1H, 17-H, J = 8.0 Hz), 4.46–4.50 q (1H, 18-H, J = 7.2 Hz), 6.11–6.16 m (1H, 3″-H_{cis}), 6.31–6.38 m (1H, 3″-H_{trans}), 7.49 d and 6.83 d (1H, $C^{15'}$=CH_2, J = 1.6 Hz), 8.03–8.10 m (1H, 3′-H), 8.81 s (1H, 20-H), 9.67 s (1H, 5-H), 9.72 s (1H, 10-H); isomer **4** (7%): –1.95 br.s and –1.84 br.s (1H each, NH), 1.70–1.74 m (3H, $C^{8''}H_3$), 1.68–1.72 m (3H, 18-CH_3), 1.98–1.78 m (2H, $C^{17''}H_2$), 2.40–2.10 m (2H, $C^{17'}H_2$), 3.04 s and 3.48 s (3H each, NCH_3), 3.32 s (3H, 7-CH_3), 3.34 s (3H, 2-CH_3), 3.47 s (3H, 12-CH_3), 3.49 s (3H, 17″-$COOCH_3$), 3.64 s (3H, 15′-$COOCH_3$), 3.76–3.84 m (2H, $C^{8'}H_2$), 4.62 br.d (1H, 17-H, J = 8.0 Hz), 4.46–4.50 m (1H, 18-H), 6.11–6.16 m (1H, 3″-H_{cis}), 6.31–6.38 m (1H, 3″-H_{trans}), 7.34 d and 6.83 d (1H each, $C^{15'}$=CH_2, J = 2.6 Hz), 8.03–8.10 m (1H, 3′-H), 8.75 s (1H, 20-H), 9.64 s (1H, 5-H), 9.67 s (1H, 10-H). Mass spectrum, m/z: 664 $[M + H]^+$, 332 $[M + H]^{2+}$, 606 $[M + H - CO_2CH_3]^+$, 592 $[M + H - CON(CH_3)_2]^+$, 532 $[M + H - CO_2CH_3 - CON(CH_3)_2]^+$, 445 $[M + H - CO_2CH_3 - CON(CH_3)_2 - CH_2CH_2CO_2CH_3]^+$.

b. Diamine **II**, 1.5 ml (11.70 mmol), was added to a solution of 100 mg (0.16 mmol) of pheophorbide *a* methyl ester (**I**) in a mixture of 10 ml of THF and 10 ml of acetic acid. The mixture was stirred for 48 h at room temperature and was then treated as described above in *a*. Yield 57 mg (45%), dark green crystals. The spectral and chromatographic parameters of the product coincided with those found for a sample obtained as described above in *a*.

c. A solution of 50 mg (0.16 mmol) of compound **IV** in a mixture of 10 ml of THF and 10 ml of acetic acid was stirred for 48 h at room temperature and was then treated as described above in *a*. Yield 22 mg (44%), dark green crystals. The spectral and chromatographic parameters of the product coincided with those found for a sample obtained as described in *a*.

This study was performed under financial support by the President of the Russian Federation (program for support of leading scientific schools; project no. NSh-4028.2008.3).

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