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Unexpected transformations of methylpheophorbide *a* under the action of bis(*N,N*-dimethylamino)methane

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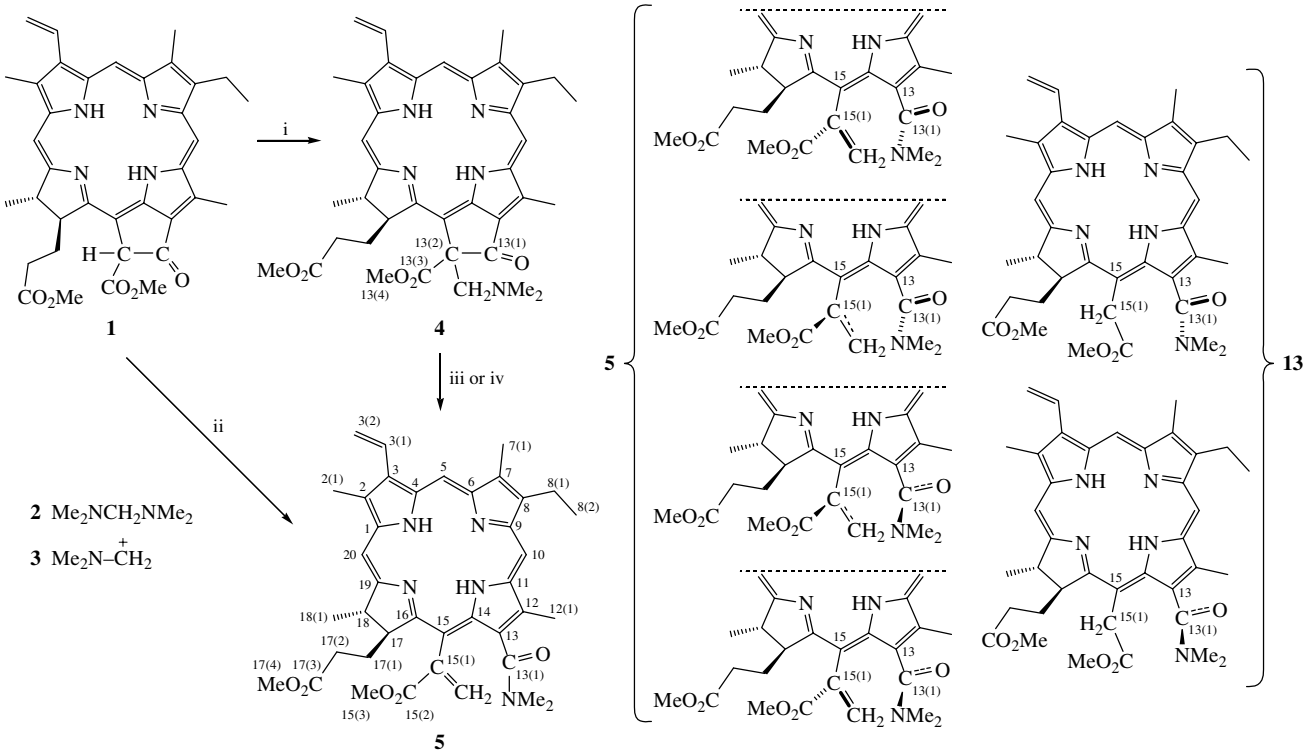
The interaction of bis(*N,N*-dimethylamino)methane with methylpheophorbide *a* leads to a chlorin e_6 13-amide derivative with a methyl acrylate fragment at the 15-position.

The chemical modifications of chlorophyll *a* derivatives, such as methylpheophorbide *a* **1** (Scheme 1), are important because the resulting chlorins are promising antitumoral agents.[1,2] Particularly, the insertion of two substituted amino groups at the periphery of a chlorin macrocycle, the subsequent alkylation of which leads to corresponding tetrasubstituted ammonium salts, is interesting for cationic photosensitizer syntheses.[3,4] It is known that bis(*N,N*-dimethylamino)methane **2** can be used as a starting material for the generation of dimethylaminomethyl cation **3**[5] (Scheme 1). Similar cation forms in the Mannich reaction. Under the action of such an electrophile on the carbonyl compounds, the formation of the corresponding dimethylaminomethyl derivatives occurs.

Here, the interaction of bis(*N,N*-dimethylamino)methane **2** with methylpheophorbide *a* **1** (Scheme 1) was studied. The reaction of **1** with an excess of **2** in THF was carried out under reflux. Under these conditions, chlorin e_6 13-amide derivative **5** with a methyl acrylate fragment at the 15-position forms instead of dimethylaminomethylation product **4** (Scheme 1).

The structure of **5** was proved by IR and NMR spectroscopy and mass spectrometry.† The valence vibration band of the C=O 13(1) keto group is absent from the IR spectrum of the product obtained because of the *exo* cycle opening, and an 'amide-I' band is present confirming the amide formation. Peaks due to single- and double-charged molecular ions and fragmentation ions are observed in the mass spectrum of the compound obtained. Note that the main direction of molecular ion fragmentation is the 13-dimethylamide group elimination, in contrast to many chlorophyll *a* derivatives, which lose the substituent at the 17-position during molecular ion fragmentation. This change in the direction of molecular ion fragmentation can be explained by the disappearance of steric hindrances resulting from 13-dimethylamide group elimination; it makes this fragmentation direction more preferable. The doublets of



Scheme 1 *Reagents and conditions*: i, **2**, THF–AcOH, 10–12 °C; ii, **2**, THF, reflux 8 h; iii, THF–AcOH, 48 h, room temperature; iv, THF, reflux.

vinyl protons in the methyl acrylate fragment and 13-*N*,*N*-dimethylamide group singlets are observed in the ^{1}H NMR spectrum of the product. An NMR study of compound **5** shows that it exists as four isomers. We suppose that atropo isomers with different positional relationship of amide group at the 13-position, methyl acrylate fragment at the 15-position and chlorin ring occur in compound **5** (Scheme 1).

The ^{1}H NMR spectrum of **5** can be interpreted as a superposition of four chlorin spectra. Each of the superposed spectra has the same amount and multiplicity of signals, and the greatest chemical shift difference of proton signals is observed for vinyl protons in methyl acrylate fragment at the 15-position and for singlets of *N*,*N*-dimethylamide group at the 13-position. Four atropo isomers can appear because of hindered rotation around the C(13)–C[13(1)] and C(15)–C[15(1)] bonds (Scheme 1). A similar phenomenon was observed for chlorin e_6 tertiary 13-amides, such as chlorin e_6 13(1)-*N*,*N*-dimethylamide-15(2),17(3)-dimethyl ester **13**: such compounds exist as two isomers differing in the 13(1)-amide group positions relatively to a chlorin ring[6,7] (Scheme 1).

A possible mechanism of the transformation of **1** into **5** is shown in Scheme 2. This mechanism was proved by the synthesis of intermediate **4** and a study of its reactions. Compound **4** can be obtained by the interaction of bis(amine) **2** with methylpheophorbide *a* **1** at 10–12 °C in a mixture of THF and acetic acid. The presence of acetic acid in reaction mixture provides cation **3** generation, whereas the low temperature allows us to avoid the isomerization of compound **4**. The structure of compound **4** was proved by IR and ^{1}H NMR spectroscopy.‡ In comparison with the ^{1}H NMR spectrum of methylpheophorbide *a* **1**, the 13(2) *exo*-ring proton singlet is absent and dimethylaminomethyl group signals are present in the spectrum of compound **4**. The valence vibration band of methene group of the dimethylaminomethyl substituent is present in the IR spectrum of the substance obtained. Mass spectrometry does not give unambiguous results. While the molecular ion peak corresponding to the structure of **4** is present in the mass spectrum, the principal fragmentation ion peaks agree with isomeric ion **5** fragmentation. Most likely, the isomerization of compound **4** occurs in the course of probe heating during mass-spectrometric measurements, and chlorin **5** molecules undergo ionization followed by fragmentation. Compound **4** also isomerises with chlorin **5** formation under refluxing in THF. Full conversion under such conditions occurs after prolonged heating (10 h) and the yield of isomerization product **5** is not high (about 20%). The isomerization of compound **4** under refluxing in THF and in mass spectrometer cell in the course of probe heating favours the formation of compound **4** as an intermediate in the reaction of bis(amine) **2** with methylpheophorbide *a* **1** (Scheme 2). The presence of a weak acid in the reaction mixture promotes the transformation of compound **4** into chlorin **5**. The isomerization can occur even at room temperature in a THF–acetic acid (1:1,

† The ^{1}H NMR spectra were recorded on a Bruker AMX-400 (400 MHz) spectrometer in $CDCl_3$. The IR spectra were recorded on a Specord M80 instrument (KBr pellets). The mass spectra were obtained using DSQ (Thermo, Direct Probe System), EIMS, 70 eV. Silica gel (La Chema, 40/100 mesh) was used for column chromatography. Methylpheophorbide *a* **1** was obtained from Spirulina;[8] its spectral data were identical to the published characteristics.[7]

Interaction of **1** with **2**. *Method A*. 1.5 ml (11.70 mmol) of **2** was added to a solution of 142 mg (0.23 mmol) of **1** in 15 ml of THF. The mixture was refluxed for 8 h. Then, it was diluted with chloroform, washed with water, dried (Na_2SO_4) and evaporated to dryness at 40–50 °C under reduced pressure. The product was purified by column chromatography on silica gel (eluent: tetrachloromethane–acetone, 50:1–1:1) and reprecipitated from chloroform–pentane to give 60 mg (40%) of compound **5** as deep green crystals. EIMS, *m*/*z*: 664 $(MH)^+$, 332 $(MH)^{+2}$, 606 $(MH - CO_2Me)^+$, 592 $(MH - CONMe_2)^+$, 532 $(MH - CO_2Me - CONMe_2)^+$, 445 $(MH - CO_2Me - CONMe_2 - CH_2CH_2CO_2Me)^+$. IR (KBr, ν/cm^{-1}): 1740, 1730 (ester $\nu_{C=O}$), 1644 (amide-I), 1606 (chlorin band), 1164, 1066 (ester C–O–C).

^{1}H NMR (400 MHz, $CDCl_3$) δ (the signals of different isomers are given separately, isomers content was determined through intensity of the proton at position 20 singlets).

Isomer 1 (47% in mixture): –2.09 (br. s, 1H, $N^{I}H$), –1.90 (br. s, 1H, $N^{III}H$), 1.72 (t, 3H, $C^{8(2)}H_3$, *J* 8.0 Hz), 1.66 (d, 3H, $C^{18(1)}H_3$, *J* 7.0 Hz), 1.98–1.78 (m, 2H, $C^{17(2)}H_2$), 2.40–2.10 (m, 2H, $C^{17(1)}H_2$), 2.55 and 3.45 (2s, 2×3H, $C^{13(1)}ONMe_2$), 3.30 (s, 3H, $C^{7(1)}H_3$), 3.42 (s, 3H, $C^{2(1)}H_3$), 3.51 (s, 3H, $C^{12(1)}H_3$), 3.50 (s, 3H, $C^{17(4)}H_3$), 3.83 (s, 3H, $C^{15(3)}H_3$), 3.76–3.84 (m, 2H, $C^{8(1)}H_2$), 4.11–4.08 (m, 1H, $C^{17}H$), 4.46 (q, 1H, $C^{18}H$, *J* 7.0 Hz), 6.14 (dd, 1H, $C^{3(2)(cis)}H$, *J* 11.3 and 1.4 Hz), 6.53 (dd, 1H, $C^{3(2)(trans)}H$, *J* 17.6 and 1.4 Hz), 7.44 and 6.49 (2d, 2×1H, $C^{15(1)}=CH_2$, *J* 0.5 and 0.5 Hz), 8.11 (dd, 1H, $C^{3(1)}H$, *J* 17.6 and 11.3 Hz), 8.88 (s, 1H, $C^{20}H$), 9.73 (s, 1H, C^5H), 9.74 (s, 1H, $C^{10}H$).

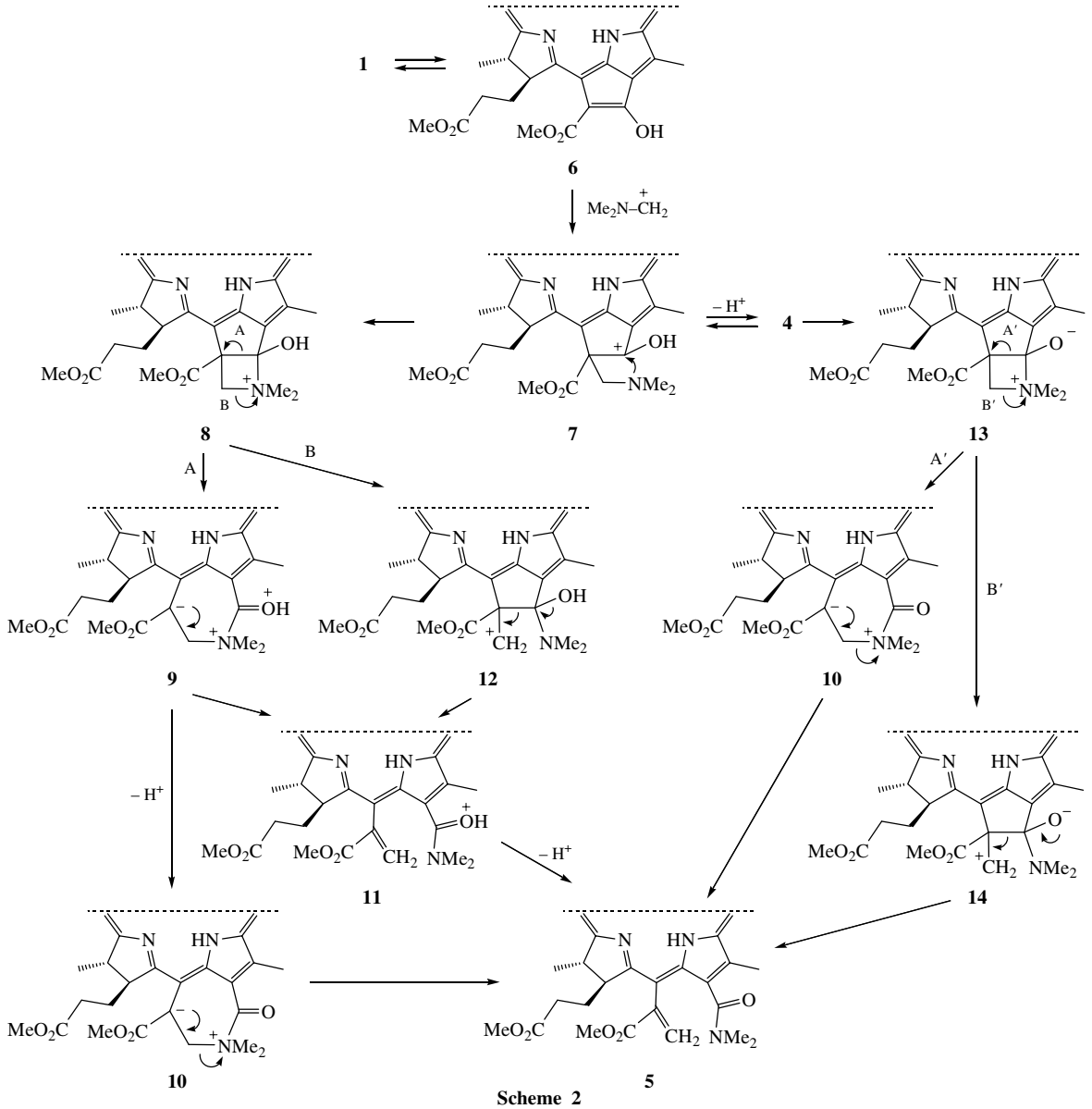
Isomer 2 (37% in mixture): –1.92 (br. s, 1H, $N^{I}H$), –1.90 (br. s, 1H, $N^{III}H$), 1.75 (t, 3H, $C^{8(2)}H_3$, *J* 7.6 Hz), 1.72 (d, 3H, $C^{18(1)}H_3$, *J* 7.6 Hz), 1.98–1.78 (m, 2H, $C^{17(2)}H_2$), 2.40–2.10 (m, 2H, $C^{17(1)}H_2$), 3.24 and 3.46 (2s, 2×3H, $C^{13(1)}ONMe_2$), 3.33 (s, 3H, $C^{7(1)}H_3$), 3.40 (s, 3H, $C^{2(1)}H_3$), 3.49 (s, 3H, $C^{12(1)}H_3$), 3.50 (s, 3H, $C^{17(4)}H_3$), 3.85 (s, 3H, $C^{15(3)}H_3$), 3.76–3.84 (m, 2H, $C^{8(1)}H_2$), 4.11–4.08 (m, 1H, $C^{17}H$), 4.47 (q, 1H, $C^{18}H$, *J* 7.6 Hz), 6.13 (dd, 1H, $C^{3(2)(cis)}H$, *J* 11.8 and 1.6 Hz), 6.53 (dd, 1H, $C^{3(2)(trans)}H$, *J* 18.0 and 1.6 Hz), 7.25 and 6.01 (2d, 2×1H, $C^{15(1)}=CH_2$, *J* 2.0 and 2.0 Hz), 8.11 (dd, 1H, $C^{3(1)}H$, *J* 18.0 and 11.8 Hz), 8.84 (s, 1H, $C^{20}H$), 9.68 (s, 1H, C^5H), 9.71 (s, 1H, $C^{10}H$).

Isomer 3 (9% in mixture): –1.73 (br. s, 1H, $N^{I}H$), –1.64 (br. s, 1H, $N^{III}H$), 1.70–1.74 (m, 3H, $C^{8(2)}H_3$), 1.62 (d, 3H, $C^{18(1)}H_3$, *J* 7.2 Hz), 1.98–1.78 (m, 2H, $C^{17(2)}H_2$), 2.40–2.10 (m, 2H, $C^{17(1)}H_2$), 2.71 and 3.46 (2s, 2×3H, $C^{13(1)}ONMe_2$), 3.32 (s, 3H, $C^{7(1)}H_3$), 3.37 (s, 3H, $C^{2(1)}H_3$), 3.48 (s, 3H, $C^{12(1)}H_3$), 3.49 (s, 3H, $C^{17(4)}H_3$), 3.55 (s, 3H, $C^{15(3)}H_3$), 3.76–3.84 (m, 2H, $C^{8(1)}H_2$), 4.67 (br. d, 1H, $C^{17}H$, *J* 8.0 Hz), 4.46–4.50 (q, 1H, $C^{18}H$, *J* 7.2 Hz), 6.11–6.16 (m, 1H, $C^{3(2)(cis)}H$), 6.31–6.38 (m, 1H, $C^{3(2)(trans)}H$), 7.49 and 6.83 (2d, 2×1H, $C^{15(1)}=CH_2$, *J* 1.6 and 1.6 Hz), 8.03–8.10 (m, 1H, $C^{3(1)}H$), 8.81 (s, 1H, $C^{20}H$), 9.67 (s, 1H, C^5H), 9.72 (s, 1H, $C^{10}H$).

Isomer 4 (7% in mixture): –1.95 (br. s, 1H, $N^{I}H$), –1.84 (br. s, 1H, $N^{III}H$), 1.70–1.74 (m, 3H, $C^{8(2)}H_3$), 1.68–1.72 (m, 3H, $C^{18(1)}H_3$), 1.98–1.78 (m, 2H, $C^{17(2)}H_2$), 2.40–2.10 (m, 2H, $C^{17(1)}H_2$), 3.04 and 3.48 (2s, 2×3H, $C^{13(1)}ONMe_2$), 3.32 (s, 3H, $C^{7(1)}H_3$), 3.34 (s, 3H, $C^{2(1)}H_3$), 3.47 (s, 3H, $C^{12(1)}H_3$), 3.49 (s, 3H, $C^{17(4)}H_3$), 3.64 (s, 3H, $C^{15(3)}H_3$), 3.76–3.84 (m, 2H, $C^{8(1)}H_2$), 4.62 (br. d, 1H, $C^{17}H$, *J* 8.0 Hz), 4.46–4.50 (m, 1H, $C^{18}H$), 6.11–6.16 (m, 1H, $C^{3(2)(cis)}H$), 6.31–6.38 (m, 1H, $C^{3(2)(trans)}H$), 7.34 and 6.83 (2d, 2×1H, $C^{15(1)}=CH_2$, *J* 2.6 and 2.6 Hz), 8.03–8.10 (m, 1H, $C^{3(1)}H$), 8.75 (s, 1H, $C^{20}H$), 9.64 (s, 1H, C^5H), 9.67 (s, 1H, $C^{10}H$).

Method B. 1.5 ml (11.70 mmol) of **2** was added to a solution of 100 mg (0.16 mmol) of **1** in a mixture of 10 ml of THF and 10 ml of acetic acid. The mixture was stirred for 48 h at room temperature. Then, the reaction mixture was treated analogously to method A to give 57 mg (45%) of compound **5** as deep green crystals. Spectral and chromatographic characteristics of the compound obtained agree with those of the sample obtained by method A.

‡ *13(2)*-N,N-*Dimethylaminomethyl-methylpheophorbide* a **4**. 1.5 ml (11.70 mmol) of **2** was added to a solution of 100 mg (0.16 mmol) of **1** in 10 ml THF and 10 ml acetic acid. The mixture was kept for 10 h at 10–12 °C. The reaction mixture was diluted with chloroform, washed with water, dried (Na_2SO_4) and evaporated to dryness at 25–30 °C under reduced pressure. The product was reprecipitated from chloroform–pentane to give 82 mg (75%) of compound **4** as deep blue crystals. IR (KBr, ν/cm^{-1}): 2876 (ν_{C-H}, CH_2NMe), 1742 (ester $\nu_{C=O}$), 1618 (chlorin band), 1164, 1044 (ester C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: –1.62 (br. s, 1H, $N^{I}H$), 0.24 (br. s, 1H, $N^{III}H$), 1.69 (t, 3H, $C^{8(2)}H_3$, *J* 7.6 Hz), 1.70 (d, 3H, $C^{18(1)}H_3$, *J* 7.2 Hz), 1.84 (s, 6H, $C^{13(2)}H_2NMe_2$), 1.87–2.21 (m, 2H, $C^{17(2)}H_2$), 2.22–2.79 (m, 2H, $C^{17(1)}H_2$), 3.24 (s, 3H, $C^{7(1)}H_3$), 3.40 (s, 3H, $C^{2(1)}H_3$), 3.53 (s, 3H, $C^{12(1)}H_3$), 3.55 (s, 3H, $C^{17(4)}H_3$), 3.72 (s, 3H, $C^{13(4)}H_3$), 3.66–3.74 (m, 2H, $C^{8(1)}H_2$), 3.99 and 4.06 (2d, 2×1H, $C^{13(2)}H_2NMe_2$, *J* 14.0 and 14.0 Hz), 4.39–4.42 (m, 1H, $C^{17}H$), 4.43 (q, 1H, $C^{18}H$, *J* 7.2 Hz), 6.17 (dd, 1H, $C^{3(2)(cis)}H$, *J* 12.0 and 1.2 Hz), 6.53 (dd, 1H, $C^{3(2)(trans)}H$, *J* 17.6 and 1.2 Hz), 8.00 (dd, 1H, $C^{3(1)}H$, *J* 17.6 and 12.0 Hz), 8.57 (s, 1H, $C^{20}H$), 9.39 (s, 1H, C^5H), 9.55 (s, 1H, $C^{10}H$).



Scheme 2

by volume) mixture. The reduction of isomerization temperature and the slight increase of product **5** yield (up to 45%) due to acid addition can be explained by the protonation of the *exo* ring carbonyl group in compound **4**, which promotes the nucleophilic attack of the 13(2)-carbon atom by the *N*,*N*-dimethylaminomethyl group. This favours the direct transformation of **7** into chlorin **5** without proton elimination, which is in accordance with the supposed mechanism. The fact that the formation and isomerisation of compound **4** are possible at relatively low temperature in a THF–acetic acid solution allows one to suggest a new synthesis of compound **5**. It can be obtained directly from methylpheophorbide *a* **1** by the reaction with bis(amine) **2** for 48 h at room temperature in a mixture of THF and acetic acid. The conditions above allow us to slightly increase the yield of chlorin **5** (up to 45%). Thus, the conditions for the synthesis of compound **4** and its chemical transformations are consistent with the mechanism suggested.

Thus, the immediate interaction of bis(*N*,*N*-dimethylamino)-methane with methylpheophorbide *a* **1** under refiuxing in THF does not lead to expected Mannich reaction product **4**. At the same time, as we found, the reaction is a simple one-step synthesis of chlorins with a 15-acryl fragment. Mannich reaction product **4** can be obtained as the result of dimethylaminomethyl cation **3** generation under the action of a weak acid (THF–AcOH mixture) at lower temperatures. The isomerization of compound **4** into chlorin **5**, as well as the synthesis of compound **5** immediately from methylpheophorbide *a* **1**, can take place under the same conditions.

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