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Facile Synthesis of 15²–Carboxamides of Methyl Pheophorbide *a*

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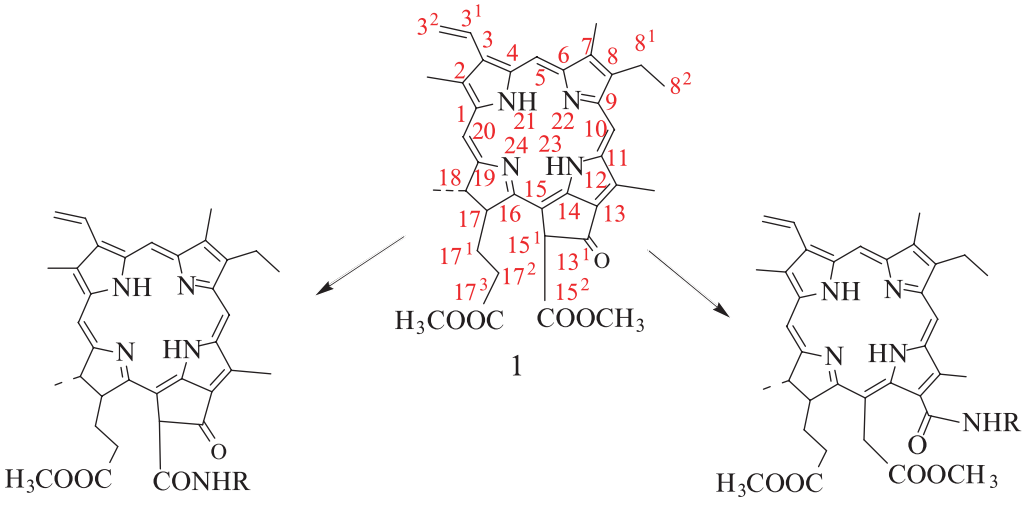
The novel synthesis of chlorin 15²-carboxamides from methyl pheophorbide, which passed without cleavage of exocycle E, is developed. The combination of this approach together with known method of opening exocycle E by amines allows easy prepartion of 13¹- and 15²-carboxamides of chlorin e_6 and methyl pheophorbide a, comprising various substituents in amide moieties. Two examples (where substituents are either benzyl-, or hexadecyl-) are presented.

Keywords: Chlorin e_6, methyl pheophorbide *a*, amidation

Tetrapyrrol macrocycles of chlorin family are widely used as optical and fluorescent sensors in various biological studies, as well as sensitizers for tumor photodynamic therapy.[1-7] Chemical modification of periphery substituents allows significantly change physical, chemical, spectral and photochemical properties of macrocycle and improve its solubility in various media, affinity to specific targets and photodynamic applications. A large number of chlorin derivatives comprising macrocycle modified with polyamines, amino acids, peptides,[8-14] carbohydrates,[15] steroids and lipids[16-18] have been synthesized and used. Therefore development of new convenient methods for introducing of substituents in various positions of chlorin macrocycle is of importance.

Herein we present a simple method for synthesis of chlorin 15²-carboxamides from available methyl pheophorbide *a*,[#] which passed without cleavage of exocycle *E*. The combination of this approach with the known method of nucleophilic opening of exocycle *E*[19] by amines allows easily prepare regioisomeric 13¹- and 15²-carboxamides of chlorin e_6 comprising various substituents in amide moieties (Scheme 1).

We discovered that interaction of methyl pheophorbide *a* **1** with 5 equivalents of amine (benzyl amine and hexadecyl amine were used) in boiling dioxane for 8 h led to 15²-carboxamides **2** and **3**, respectively.[♣] The presence of exocyclic ring in compounds **2** and **3** was unequivocally demonstrated by absorption spectra (Figure 1), as well as by ^{13}C NMR spectra, displayed resonances characteristic for carbonyl groups (192.06 ppm and 192.50 ppm for compounds **2** and **3**, respectively). The yield of target products **2** and **3** in this reaction exceeds 80 %; besides we have found chlorin derivatives comprising opened exocyclic ring (8-11 %). These by-products were identified as 13¹-carboxamides



2, R= -$CH_2C_6H_5$
3, R= -$(CH_2)_{15}CH_3$

4, R= -$CH_2C_6H_5$
5, R= -$(CH_2)_{15}CH_3$

Scheme 1.

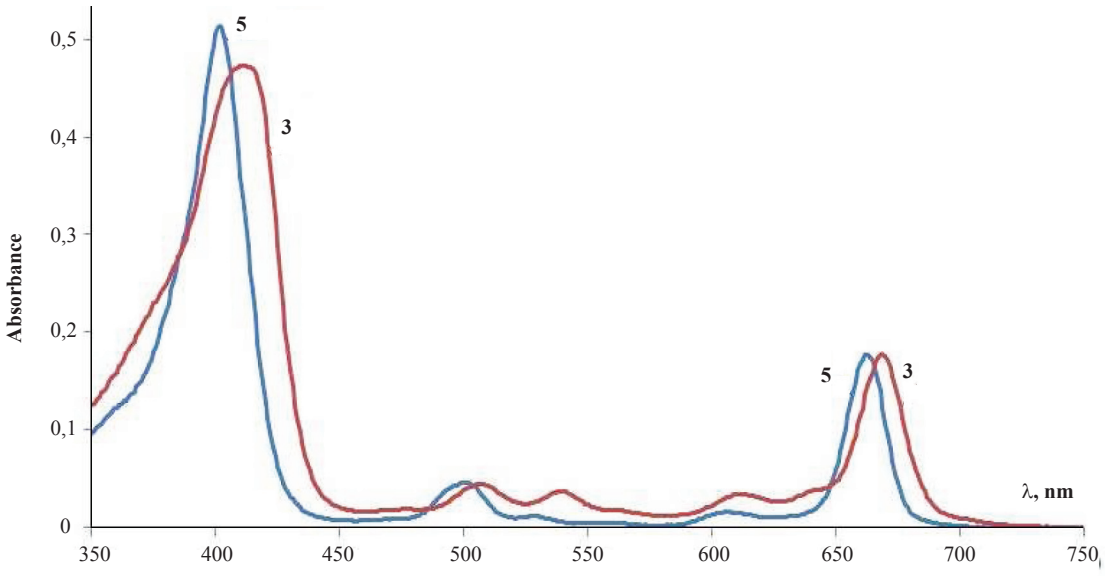


Figure 1. Absorption spectra of compounds **3** and **5** in CH_2Cl_2 (spectra of compounds **2** and **4** were identical to those for related counterparts).

4 and **5**♣ (identification was carried out by comparison of their HRMS, ^{1}H NMR and absorption spectra with those for authentic samples **4** and **5** prepared according to reported methods [20-22]).

We concluded that nucleophilic substitution of 15^2-methyl ester for amine and nucleophilic opening of exocyclic ring by amine in methyl pheophorbide *a* **1** are independent. The use of non volatile amines such as benzyl amine and hexadecyl amine, high temperature of reaction, and effect of β-carbonyl group in methyl pheophorbide *a* **1** promote formation of 15^2- carboxamide, which proceeds without exocyclic ring cleavage. On the other hand, prolong reaction at a relatively low temperature (THF, 35-40^{o}C, 14-72 h, depending on the structure of amine used) is in favor to nucleophilic opening of exocyclic ring.

Exocyclic ring in 15^2-carboxamides **2** and **3** was more resistant to nucleophilic opening by amines in comparison with that in methyl pheophorbide *a* **1**. Independent experiment showed that incubation of 15^2-carboxamide **3** with benzyl amine in THF at 40^{o}C for 72 h resulted in only traces of related 13^1,15^2-dicarboxamide (< 3%). This difference in reactivity of exocycle ring *E* in compounds **1** and **3** towards amines was apparently due to different electron withdrawing effects of either ester, or amide groups.

All indicated compounds **2-5** were isolated in pure forms, their structures were fully confirmed by HRMS, ^{1}H NMR, ^{13}C NMR and absorption spectra. Taken together, data presented above revealed that reaction of methyl pheophorbide *a* **1** with amines allows simple preparation of either 13^1-caboxamides with opened exocycle *E*, or 15^2-carboxamides with saved exocycle *E*, depending on reaction conditions. The choice of conditions is very important: recent publications[22,23] revealed that reaction of methyl pheophorbide *a* **1** with secondary amines in boiling toluene for 1.5-2 h led to mixtures of related 13^1-carboxamides with opened exocylce *E* and 15^2-carboxamides with saved exocycle *E*.

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Notes and References

Absorption spectra were registered with a 'Thermospectronic Helios α' spectrophotometer in CH_2Cl_2; ^{1}H NMR and ^{13}C NMR spectra - with an 'AMX-III' 400 MHz Bruker instrument in $CDCl_3$; high resolution mass spectra (HMRS) – with a Bruker 'Apex Ultra' FT ICR MS instrument at ion positive electro spray ionization mode. Flash chromatography was performed on silica gel G (0.015- 0.040 mm), analytical TLC – on UV254-HPTLC silica gel plates, preparative TLC – on UV254-PTLC silica gel plates ''Merck''; methyl pheophorbide *a* **1** was isolated from *Spirulina platencis.*

♣ *Methyl 15^2-(benzylcarbamoyl)pheophorbide a,* **2.** HRMS, calculated for $[C_{42}H_{44}N_5O_4]^+$: 682.3393, found: 682.3378. ^{1}H NMR δ_H ppm: 8.56, 9.38, 9.51 (each 1H, s, 5-, 10-, 20-H), 7.99 (1H, dd, J = 11.6 Hz and J = 17.8 Hz, 3^1-H), 7.42 (2H, d, J = 7.4 Hz, *o*-Ph), 7.33 (2H, t, J = 7.4 Hz, *m*-Ph), 7.26 (1H. t, J = 7.4 Hz, *p*-Ph), 6.28 (1H, dd, J = 17.8 Hz and J = 1.5 Hz, 3^2-H, *cis*), 6.17 (1H, dd, J = 11.6 Hz, J = 1.5 Hz, 3^2-H, *trans*), 4.67 (2H, m, CH_2Ph), 4.45 (2H, dt, J = 8.7 Hz and J = 2.0 Hz, 17^1-H), 4.38 (1H, qd, J = 7.2 Hz, J = 2.0 Hz, 8^1-H), 3.23, 3.40, 3.55, 3.68 (each 3H, s, 2-, 7-, 12- and O-CH$_3$), 2.20-2.34 (2H, m, 17^2-H), 1.81 (3H, d, J = 7.3 Hz, 18-CH$_3$); 1.70 (3H, t, J = 7.2 Hz, 8^2-CH$_3$), -1.60 (1H, s, NH). ^{13}C NMR δ_C ppm: 11.25; 12.09; 12.14; 17.40; 19.48; 23.07; 29.97; 30.92; 44.19; 50.25; 51.20; 51.68; 65.44; 93.24; 97.47; 104.29; 105.31; 122.71; 127.41; 127.96; 128.58; 128.69; 128.92; 129.16; 136.13; 136.27; 136.55; 137.95; 138.47; 138.75; 141.98; 145.19; 149.93; 150.95; 155.64; 162.73; 167.52; 172.26; 173.74; 192.06. UV-vis λ_{max} nm (ε): 411 (96300); 504 (8200); 535 (7000); 604 (5200); 668 (35300).

Methyl 15^2-(hexadecylcarbamoyl)pheophorbide a, **3.** HRMS, calculated for $[C_{51}H_{70}N_5O_4]^+$: 816.5428, found: 816.5445. ^{1}H NMR δ_H ppm: 8.56, 9.38, 9.50 (each 1H, s, 5-, 10-, 20-H), 7.99 (1H, dd, J = 11.6 Hz and J = 17.8 Hz, 3^1-H); 6.95 (1H, t, J =

7.0 Hz, NH); 6.28 (1H, dd, J = 17.8 Hz and J = 1.5 Hz, 3²-H, *cis*); 6.16 (1H, dd, J = 11.6 Hz, J = 1.5 Hz, 3²-H, *trans*); 4.55 (2H, dt, J = 8.7 Hz and J = 2.0 Hz, 17¹-H); 4.40 (1H, qd, J = 7.2 Hz, J = 2.0 Hz, 8¹-H); 3.23, 3.39, 3.56, 3.67 (each 3H, s, 2-, 7-, 12- and O-CH_3); 2.21-2.34 (2H, m, 17²-H); 1.88 (3H, d, J = 7.3 Hz, 18-CH_3); 1.70 (3H, t, J = 7.2 Hz, 8²-CH_3); 1.25 (br., CH_2 in hexadecyl moiety); 0.86 (3H, as. t, CH_3 in hexadecyl moiety); -1.60 (br. s, NH). ^{13}C NMR δ_C ppm: 11.32; 12.16; 14.17; 17.47; 19.58; 22.77; 23.21; 23.80; 29.44; 29.64; 29.69; 29.74; 29.77; 29.85; 30.12; 31.05; 32.01; 37.14; 38.70; 40.38; 50.33; 51.32; 51.71; 53.48; 65.41; 67.92; 93.33; 97.52, 104.32; 105.56; 122.76; 128.70; 128.94; 129.28; 130.95; 131.92; 136.18; 136.22; 136.34; 136.66; 138.03; 142.05; 145.26; 149.98; 151.02; 155.72; 163.08; 167.35; 172.37; 173.80; 192.50. UV-vis λ_{max} nm (ε): 411 (96300); 506 (8400); 539 (7300); 611 (5700); 669 (35900).

♠ *13¹-(Benzylcarbamoyl)chlorin,* **4.** HRMS, calculated for $[C_{43}H_{48}N_5O_5]^+$: 714.8845; found: 714.8852. ^{1}H NMR δ_H ppm: 8.80, 9.61, 9.86 (each 1H, s, 5-, 10-, 20-H); 8.05 (1H, dd, J = 11.6 Hz and J = 17.8 Hz, 3¹-H); 7.55 (2H, d. J = 7.4 Hz, *o*-Ph); 7.42 (2H, t, J = 7.4 Hz, *m*-Ph); 7.34 (1H. t, J = 7.4 Hz, *p*-Ph); 6.81 (1H, t, J = 7.0 Hz, NH); 6.33 (1H, dd, J = 17.8 Hz and J = 1.5 Hz, 3²-H, *cis*); 6.12 (1H, dd, J = 11.6 Hz, J = 1.5 Hz, 3²-H, *trans*); 5.28 and 5.55 (each 1H, d, J = 18.9 Hz, 15¹-H); 4.78 (1H, dd, J = 5.0 Hz and J = 14.5 Hz, 17²-H); 5.06 (1H, dd, J = 6.1 Hz and J = 14.5 Hz, 17²-H); 4.47 (2H, m, CH_2Ph); 4.39 (1H, qd, J = 7.2 Hz, J = 2.0 Hz, 8¹-H); 3.29, 3.48, 3.53, 3.62, 3.72 (each 3H, s, 2-, 7-, 12- and O-CH_3); 2.22-2.35 (2H, m, 17²-H); 1.72 (3H, d, J = 7.3 Hz, 18-CH_3); 1.71 (3H, t, J = 7.2 Hz, 8²-CH_3); -1.77 (s, NH). ^{13}C NMR δ_C ppm: 11. 35; 12.03; 12.18; 17.72; 19.72; 23.09; 23.91; 29.15; 29.82; 31.24; 38.03; 44.93; 49.34; 51.63; 52.19; 53.19; 67.66; 93.71; 98.90; 101.46; 107.94; 121.64; 127.84; 128,36; 128.96; 129.53; 129.96; 130.24; 134.64; 134.93; 135.03; 135.08; 136.13; 137.99; 138.99; 144.82; 149.16; 154.33; 166.72; 168.91; 169.37; 173.55; 174.02. UV-vis λ_{max} nm (ε): 402 (104000); 500 (9100); 528 (2000); 607 (3000); 663 (35800).

13¹-(Hexadecylcarbamoyl)chlorin, **5.** HRMS, calculated for $[C_{52}H_{74}N_5O_5]^+$: 848.5690; found: 848.5673. ^{1}H NMR δ_H ppm: 8.80, 9.63, 9.69 (each 1H, s, 5-, 10-, 20-H); 8.08 (1H, dd, J = 11.6 Hz and J = 17.8 Hz, 3¹-H); 6.40 (1H, t, J = 7.0 Hz, NH); 6.35 (1H, dd, J = 17.8 Hz and J = 1.5 Hz, 3²-H, *cis*); 6.13 (1H, dd, J = 11.6 Hz, J = 1.5 Hz, 3²-H, *trans*), 4.55 (dt, J = 8.7 Hz and J = 2.0 Hz, 17¹-H); 4.40 (1H, qd, J = 7.2 Hz, J = 2.0 Hz, 1H, 8¹-H); 3.31, 3.48, 3.56, 3.60, 3.80 (each 3H, s, 2-, 7-, 12- and O-CH_3); 2.21-2.34 (2H, m, 17²-H); 1.72 (3H, d, J = 7.3 Hz, 18-CH_3); 1.74 (3H, t, J = 7.2 Hz, 8²-CH_3); 1.25 (br., CH_2 in hexadecyl moiety); 0.88 (3H, as. t, CH_3 in hexadecyl moiety); -1.79 (br. s, NH). ^{13}C NMR δ_C ppm: 11.42; 12.08; 12.22; 14.19; 17.68; 19.80; 22.78; 23.16; 23.90; 27.34; 29.21; 29.46; 29.61; 29.69; 29.72; 29.76; 29.79, 29.85; 30.12; 31.24; 32.02; 32.90; 37.15; 37.99; 40.85; 48.14; 49.42; 50.10; 51.65; 52.20; 53.30; 53.48; 63.12; 93.12; 94.14; 98.90; 101.38; 102.67; 121.94; 128.84; 130.10; 134.93; 135.35; 135.93; 139.28; 144.53; 149.20; 153.50; 167.32; 169.29; 173.56; 174.11. UV-vis λ_{max} nm (ε): 402 (104000); 500 (9100); 528 (2000); 607 (3000); 663 (35800).

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