Facile Synthesis of $15^2$–Carboxamides of Methyl Pheophorbide $a$

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The novel synthesis of chlorin $15^2$-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 preparation of $13^1$- and $15^2$-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 $15^2$-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 $13^1$- and $15^2$-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 $15^2$-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 $13^1$-carboxamides

The synthesis of $15^2$-carboxamides was accomplished in a few steps. The interaction of methyl pheophorbide $a$ 1 with 5 equivalents of amine in boiling dioxane for 8 h led to the formation of carboxamides 2 and 3, respectively. The presence of exocyclic ring in compounds 2 and 3 was confirmed by spectroscopic methods, including absorption and NMR spectra. The yield of target products 2 and 3 in this reaction was found to exceed 80%. Furthermore, the formation of by-products containing opened exocyclic ring was observed, indicating the potential for further manipulation of the macrocycle.

Scheme 1.
4 and 5* (identification was carried out by comparison of their HRMS, ¹H 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 15²-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 15²-carboxamide, which proceeds without exocyclic ring cleavage. On the other hand, prolong reaction at a relatively low temperature (THF, 35-40 °C, 14-72 h, depending on the structure of amine used) is in favor to nucleophilic opening of exocyclic ring.

Exocyclic ring in 15²-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 15²-carboxamide 3 with benzyl amine in THF at 40 °C for 72 h resulted in only traces of related 13¹,15²-dicarboxamide (< 3%). This difference in reactivity of exocyclic 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, ¹H NMR, ¹³C NMR and absorption spectra. Taken together, data presented above revealed that reaction of methyl pheophorbide a 1 with amines allows simple preparation of either 13¹-carboxamides with opened exocycle E, or 15²-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 13¹-carboxamides with opened exocycle E and 15²-carboxamides with saved exocycle E.

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**Notes and References**

Absorption spectra were registered with a ‘Thermospectronic Helios α’ spectrophotometer in CH₂Cl₂; ¹H NMR and ¹³C NMR spectra - with an ‘AMX-III’ 400 MHz Bruker instrument in CDCl₃; high resolution mass spectra (HRMS) – 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 “Merek”; methyl pheophorbide a 1 was isolated from Spirulina platensis.

**Methyl 15²-(benzylcarbamoyl)pheophorbide a, 2.** HRMS, calculated for [C₄₂H₄₄N₅O₄]⁺: 682.3393, found: 682.3378. ¹H NMR δ 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, 3¹-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, 3²-H, cis), 6.17 (1H, dd, J = 11.6 Hz, J = 1.5 Hz, 3²-H, trans), 4.67 (2H, m, CH₂Ph), 4.45 (2H, dt, J = 8.7 Hz and J = 2.0 Hz, 17⁻-H), 4.38 (1H, d, J = 7.2 Hz, J = 2.0 Hz, 8⁻-H), 3.23, 3.40, 3.55, 3.68 (each 3H, s, 2-, 7-, 12- and O-CH₃), 2.20-2.34 (2H, m, 17⁻-H), 1.81 (3H, d, J = 7.3 Hz, 18-CH₃); 1.70 (3H, t, J = 7.2 Hz, 8⁻-CH₃), -1.60 (1H, s, NH). ¹³C NMR δ ppm: 112.25; 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; 131.20; 131.63; 132.17; 132.65; 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, nm (ε): 411 (96300); 504 (8200); 535 (39800); 590 (1000); 604 (5200); 668 (353000).

**Methyl 15²-(hexadecylcarbamoyl)pheophorbide a, 3.** HRMS, calculated for [C₅₁H₇₀N₅O₄]⁺: 816.5428, found: 816.5445. ¹H NMR δ 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, 3¹-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, 3²-H, cis), 6.17 (1H, dd, J = 11.6 Hz, J = 1.5 Hz, 3²-H, trans), 4.67 (2H, m, CH₂Ph), 4.45 (2H, dt, J = 8.7 Hz and J = 2.0 Hz, 17⁻-H), 4.38 (1H, d, J = 7.2 Hz, J = 2.0 Hz, 8⁻-H), 3.23, 3.40, 3.55, 3.68 (each 3H, s, 2-, 7-, 12- and O-CH₃), 2.20-2.34 (2H, m, 17⁻-H), 1.81 (3H, d, J = 7.3 Hz, 18-CH₃); 1.70 (3H, t, J = 7.2 Hz, 8⁻-CH₃), -1.60 (1H, s, NH). ¹³C NMR δ ppm: 112.25; 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; 131.20; 131.63; 132.17; 132.65; 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, nm (ε): 411 (96300); 504 (8200); 535 (7000); 604 (5200); 668 (353000).

Figure 1. Absorption spectra of compounds 3 and 5 in CH₂Cl₂ (spectra of compounds 2 and 4 were identical to those for related counterparts).

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7.0 Hz, NH); 6.28 (1H, dd, J = 17.8 Hz and J = 1.5 Hz, 3'-H, cis); 6.16 (1H, dd, J = 11.6 Hz, J = 1.5 Hz, 3'-H, trans); 4.55 (2H, dt, J = 8.7 Hz and J = 2.0 Hz, 17'-H); 4.40 (1H, qd, J = 7.2 Hz, J = 2.0 Hz, 8'-H); 3.23, 3.39, 3.56, 3.67 (each 3H, s), 2.7-, 12- and O-CH3); 2.21-2.34 (2H, m, 17'-H); 1.88 (3H, d, J = 7.3 Hz, 18-CH3); 1.70 (3H, J = 7.2 Hz, 8'-CH3); 1.25 (br, CH; in hexadecyl moiety); 0.86 (3H, as. t, CH, in hexadecyl moiety); -1.60 (br. s, NH). 13C NMR δ ppm: 131.33; 135.33; 136.13; 137.99; 138.99; 144.82; 149.16; 153.48; 155.71; 156.52; 159.38; 167.71; 172.37; 173.80; 192.50. UV-vis λmax nm (ε): 715 (35800), 718 (35900).

References


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