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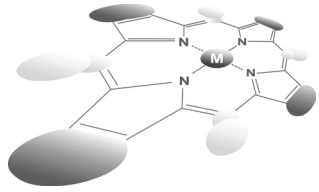

Functionalization of chlorin e_6 trimethylester towards potential amphiphilic photosensitizers for photodynamic therapy

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ABSTRACT: Chlorins (dihydroporphyrins) are considered, due to their ideal photophysical properties, as attractive photosensitizers for photodynamic therapy (PDT) of cancer and other therapeutic and diagnostic applications. Chlorophyll *a*, as a naturally occurring chlorin, forms an almost unlimited renewable resource for preparation of potential biologically active chlorin photosensitizers and fluorescence markers. To achieve amphiphilic photosensitizers which might be selectively enriched in tumor cells, we addressed linkage of *per se* lipophilic chlorophyll derivatives with carbohydrate based hydrophilic aminopolyols.

KEYWORDS: chlorin e_6, aminopolyols, photosensitizer, PDT, spirulina.

INTRODUCTION

Nature provides chlorophyll *a* **1** as a pigment of photosynthesis, the most prominent and widespread representative among the class of natural and artificial chlorins (Fig. 1) [1, 2]. Plants produce mixtures of chlorophyll *a* and *b* as pigments of photosynthesis. Isolation of pure chlorophyll *a* requires chromatographic separations. In contrast, cyanobacteria like *Spirulina platensis* form only chlorophyll *a* **1** as a photosynthesis pigment. The possibility to obtain only chlorophyll *a* from *Spirulina* and widespread cultivation plants for *Spirulina* in subtropical and tropical areas around the Pacific Ocean form an ideal renewable feedstock for production of chlorophyll *a*-derived chlorin photosensitizers [3,4]. Based on previous procedures for isolation of chlorophyll *a* from *Spirulina*, we improved the extraction technique and made methyl pheophorbide *a* **2** available on a larger scale. Transformation of methyl pheophorbide *a* **2** into chlorin e_6 trimethlylester **3** (Chl e_6 tme) provides a robust key intermediate for further functionalization [2b]. To date, studied chlorophyll *a* derived amphiphilic photosensitizers like mono-L-aspartyl chlorin e_6 were linked with additional hydrophilicity generating carboxylic acid functions to achieve overall amphiphilicity. Transport and tissue enrichment of this type of photosensitizers are substantially influenced by pH values of biological targets [5]. Amphiphilic chlorins with pH-independent hydrophilic moieties should overcome the problem of pH influence. In cell experiments with diglucosylated artificial chlorins, we demonstrated advantages of these "neutral" amphiphilic photosensitizers [6].

◊SPP full member in good standing

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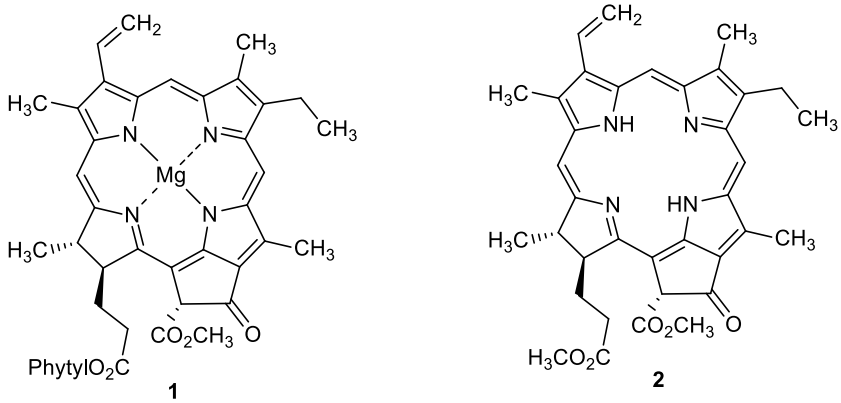
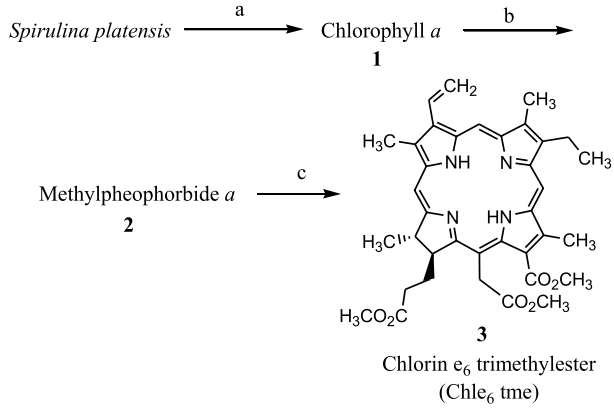


Fig. 1. Chlorophyll *a* **1** and its direct transformation product methyl pheophorbide *a* **2**

RESULTS AND DISCUSSION

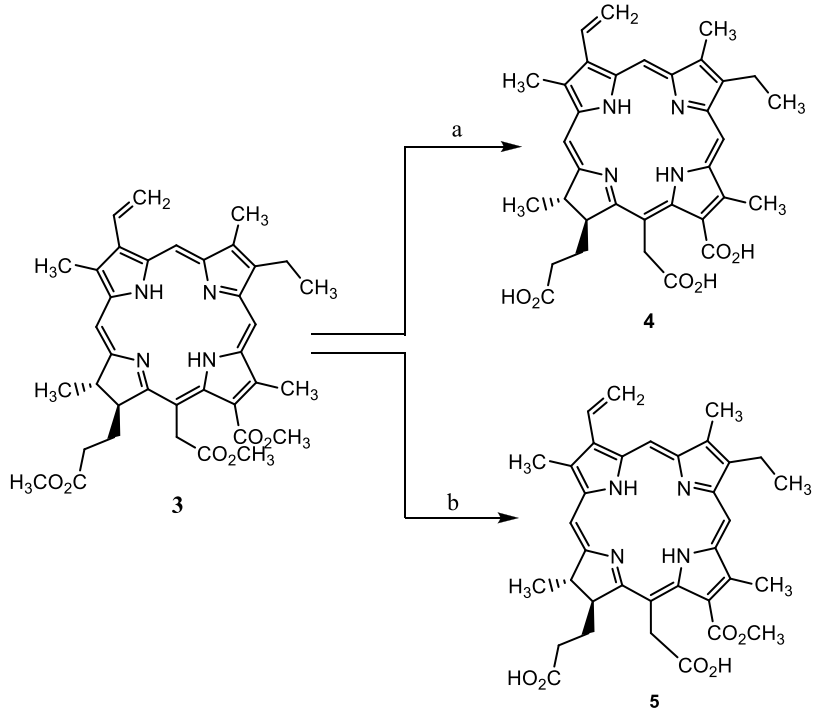
Spirulina platensis which was cultivated in Vietnam proved to be an effective source for extraction of pure chlorophyll *a* **1**. It was then further transformed *via* methyl pheophorbide *a* **2** into chlorin e_6 trimethyl ester **3**. Prior to extraction, the chlorophyll *a* content of *Spirulina* biomass was determined by a procedure reported by Lichtenthaler for determination of chlorophyll content of higher plants [7]. Only *Spirulina* biomass batches of more than 0.4% chlorophyll content were considered for the extraction procedure. With Soxhlet thimbles of 325 mm length and 80 mm in diameter, 700 g of *Spirulina* biomass could be extracted to yield 4.2 g of methyl pheophorbide *a* **2** according a 0.6% chlorophyll *a* content (Scheme 1). Prior to extraction, the biomass was filled into the Soxhlet thimble. The filled thimble was then placed into a Dewar container of appropriate size, treated with liquid nitrogen and subsequently transferred to the Soxhlet extractor. The procedure simplifies manipulation of the biomass.



Scheme 1. Extraction of chlorophyll *a* **1** from *Spirulina platensis* and its transformation into chl e_6 tme **3**. (a) (i) Dry spirulina mass, acetone, N_2 (liq.), *ca.* 60 min, then warm up to rt; (ii) acetone, reflux, 24 h. (b) H_2SO_4 conc., MeOH, Ar, rt, 20 h. (c) 1 M KOH in MeOH, THF/$CHCl_3$, Ar, rt, 30 min (73%) or KOMe, MeOH, acetone, Ar, rt, 30 min, (85%)

As expected and determined by HPLC and ^{1}H-NMR spectroscopy (see Supporting information), methyl pheophorbide *a* **2** consisted of a mixture of $13^2R/13^2S$ diastereomers in a 89:11 ratio. The mixture is formed in a deprotonation–protonation equilibrium of the β-ketoester moiety of isocyclic ring E. In a *retro*-Claisen condensation, methyl pheophorbide *a* **2** was transformed into chlorin e_6 trimethylester [2b,8]. The reaction was performed with potassium hydroxide in MeOH/THF/$CHCl_3$ to give chlorin e_6 trimethylester **3** in 73% yield. A higher yield of 85% could be achieved with potassium methoxide in MeOH/acetone. On a larger scale the first variant might be easier to perform. With chlorin e_6 trimethyl ester **3**, a robust crystalline key intermediate is available for further transformations. In numerous studies chlorin e_6 **4** was used for functionalization with respect to preparation of sensitizers for PDT [5].

Alkaline hydrolysis of chl e_6 tme **3** yielded chlorin e_6 **4** in high yield (Scheme 2). However, for pharmaceutical applications chlorin e_6 of high purity is required. To achieve a certain purity of chlorin e_6 **4** it is necessary to perform a reversed phase chromatography which is cost-intensive on a technical scale. To avoid reversed phase chromatography we considered preparation of chlorin e_6 13-monomethylester **5** as an alternative. Preparation of chlorin e_6 monomethylester **5** among different di- and mono-esters of chlorin e_6 was reported by Conant and Armstrong. [9]. Conant's work demonstrated that a chlorin e_6 monomethylester could be formed by alkaline hydrolysis from chl e_6 tme **3** in low yield in a mixture with chl e_6 **4** and other components. An unequivocal structure determination of Conant's chlorin e_6 monomethylester is missing due to at-that-time unavailable methods for structure elucidation. In our case, hydrolysis of chl e_6 tme **3** gave chlorin e_6 13-monomethylester **5** in high yield under acidic reaction conditions. Without any chromatographic purification the crude reaction product could be



Scheme 2. Formation of chl e_6 **4** and chl e_6 mme **5** by selective hydrolysis. (a) KOH (15%) in MeOH, acetone, Ar, 40 °C, 40 min, then reflux, 2.5 h (99%). (b) 5 M HCl, H_2O, 90 °C, 30 min (82%)

crystallized isothermally from $CHCl_3$/ petroleum ether to give chl e_6 mme **5** of high purity. Comprehensive $^1H,^1H$-homonuclear NMR- (COSY-, NOESY-correlations) and $^1H,^{13}C$-heteronuclear NMR-spectra (HSQC-, HMBC-correlations) of chl e_6 mme **5** (for details see Supporting information) revealed unambiguously its constitution with a methylester group in the 13 position.

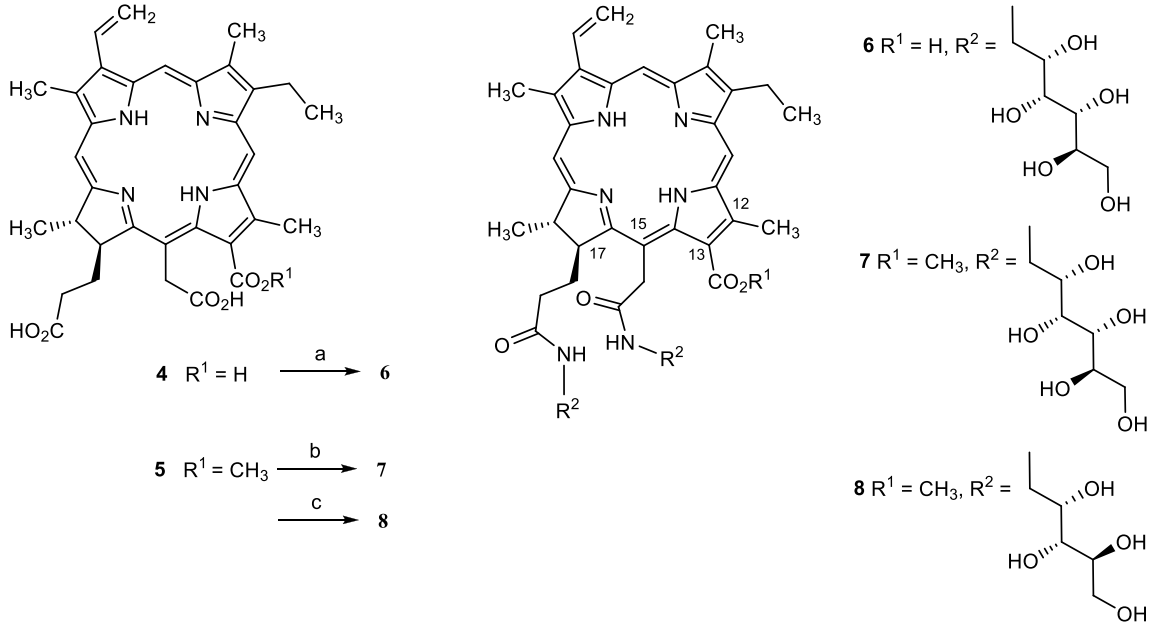
The harsh alkaline and prolonged reaction conditions for hydrolysis of chlorin e_6 trimethelester **3** cleaved all three ester groups to yield chlorin e_6 **4**, whereas under carefully optimized acidic reaction conditions, only 15 and 17 ester functions were hydrolyzed to yield chlorin e_6 13-mono methylester **5** selectively. The reactivity of the 13 carboxylic group is significantly reduced by its conjugation with the electron donating chlorin chromophore.

With chlorin e_6 **4** and chlorin e_6 mme **5** in hand, the synthesis of amphiphilic chlorin derivatives was envisaged (Scheme 3). Commercially available glucamine and L-1-amino-1-deoxy-arabitinol were chosen as hydrophilic moieties to be linked to chlorin chromophores. L-1-amino-1-deoxy-arabitinol was prepared from L-arabinose according to a literature procedure [10]. After activation of free carboxylic acid groups of chl e_6 **4** and chl e_6 mme **5,** the subsequent reaction with glucose- and arabinose-based aminopolyols formed chlorin diamides **6**, **7** and **8**. Linkage between chlorins and polyols *via* amide bonds should stabilize the amphiphilic sensitizers towards possible hydrolysis in biological environments. Chlorin e_6 **4** yielded 15^2, 17^4-diamide, which could be expected due to the reduced reactivity of 13-carboxylic acid function. A similar observation was also made before [11].

CONCLUSION

Solubility of amphiphilic photosensitizers in water plays a crucial role for their intravenous application as aqueous solutions. Though chlorin polyoldiamides **7** and **8** are not soluble in pure water, solubility in aqueous solution could be achieved by addition of 0.3% DMSO. For medical applications this does not present any problems because DMSO supplements are quite common in medicine. In contrast to chlorin diamides **7** and **8**, chlorin e_6 polyoldiamide **6** exhibits good solubility in pure water, obviously due to the free 13-carboxylic acid group.

The absorption spectrum of chlorin e_6 13-methylester-15^2,17^3-glucodiamide **7** (Fig. 2) shows the Soret band at 401 nm and the long wavelength Q_y band at 663 nm. The spectrum of **7** is also characteristic for the other chlorins which were investigated here. Comprehensive photophysical studies were performed and are reported elsewhere [12,13]. The most important photophysical properties for PDT in addition to long wavelength absorption are fluorescence and singlet oxygen formation for tumor localization and tumor destruction respectively. For Chl e_6 mme **5**, Chl e_6 13-methylester 15^2,17^3-glucodiamide **7** and Chl e_6 13-methylester-15^2,17^3-arabodiamide **8**, wavelength of fluorescence, quantum yields of fluorescence (Φ_f) and singlet oxygen formation (Φ_Δ) are summarized in Table 1.



Scheme 3. Formation of polyol amide derivatives of chl e_6 **4** and chl e_6 mme **5**. (a) (i) *i*BuOCOCl, NEt_3, THF, -15 °C, *ca.* 15 min; (ii) glucamine, NEt_3, $EtOH/H_2O$, rt, 15 h (88%). (b) (i) *i*BuOCOCl, NEt_3, THF, -15 °C, 1 h; (ii) glucamine, NEt_3, $EtOH/H_2O$, rt, 15 h (59%). (c) (i) *i*BuOCOCl, NEt_3, THF, -15 °C, 30 min; (ii) 1-amino-1-deoxy-arabitinol, NEt_3, $EtOH/H_2O$, rt, 15 h (61%)

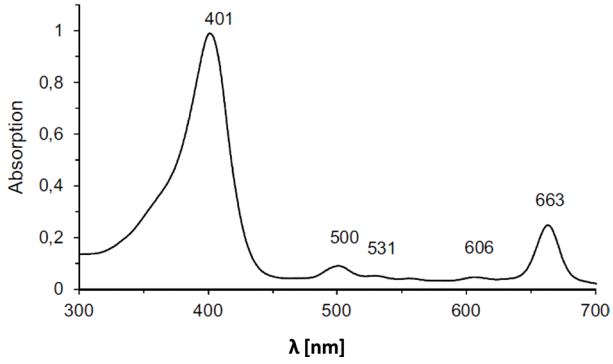


Fig. 2. UV-vis spectrum of chlorin e_6 13-methylester-15^2,17^3-diglucamide **7** in MeOH

As required for PDT photosensitizers, chlorins **5**, **7** and **8** show fluorescence at the Q_y band and exhibit sufficient values for quantum yield of singlet oxygen formation of *ca.* $\Phi_\Delta = 0.5$.

Preliminary experiments with human colon adenocarcinoma cells (HCT116p53) demonstrate IC_{50} values for phototoxicity of 0.22+/-0.03 μMol for chl e_6 mme diarab **8** incubation followed by irradiation with red light (20 J/cm^2) [12].

EXPERIMENTAL

General

Starting materials were either prepared according to literature procedures or were purchased from Fluka, Merck, Acros Organics or Sigma Aldrich and used without further purification. All solvents were purified and dried by standard methods. All reactions were carried out under argon. Melting points are not corrected. TLC: Silica gel plates (Macherey & Nagel, Polygram SIL G/UV_{254}) and aluminum oxide plates (Macherey & Nagel, Polygram Alox N/UV_{254}). Column chromatographic separations were performed on silica gel (32–63 μm, 60 Å, ICN), aluminum oxide (activity grade II–III, neutral, ICN), Matrix silica gel (20–45 μm, 60 Å, Amicon) and silica gel 100, C18-reversed phase (0.015–0.035 mm, Fluka). HPLC: Knauer HPLC instrument with pump 64, two-channel potentiometer

Table 1. Selected photophysical data of chlorin photosensitizers **5**, **7** and **8**

		λ_f^{em} [nm]	Φ_f	Φ_Δ	Ref.
5	*Chl e_6 mme*	664 (MeCN)	0.14 (MeCN)	0.6 (CH_2Cl_2)	[11]
7	*Chl e_6 mme diglu*	666 (MeCN)	0.04 (MeCN)	0.5 (CH_2Cl_2)	[11]
8	*Chl e_6 mme diarab*	674 (DMSO)	0.25 (EtOH)	0.63 (EtOH)	[12]

BBC Metrawatt Servogor 120 recorder and Knauer UV spectrometer. UV-Vis: Varian Cary 50 spectrophotometer and HP 8453 spectrophotometer diode array detection. IR: Perkin–Elmer Paragon 500 FT-IR spectrometer. NMR spectra: Bruker DPX-200 AVANCE, Bruker AM 360 spectrometer and Bruker AMX 600 spectrometer. All chemical shifts were referenced to TMS lock signal. Exact assignment of proton signals in ^{1}H NMR spectra was achieved by two dimensional COSY, NOESY, HSQC and HMBC experiments. MS: EI: Finnigan MAT 8222 [E (70 eV), 200 °C] and ESI: Bruker ESQUIRE-LC [3.8kV, 2 µL/min].

Isolation of methyl pheophorbide a* 2 *as an* 89:11 *mixture of* 13^2R:13^2S *diastereomers from Spirulina platensis. Dry spirulina biomass (700 g) was filled into a Soxhlet thimble (length 32.5 cm, diameter 8 cm) and placed in a Dewar container of appropriate size. (To ensure effective production of methyl pheophorbide *a*, only spirulina biomass batches of more than 0.4% chlorophyll content were considered for extractions.) Therefore prior to extraction, the chlorophyll *a* content of the spirulina biomass was determined according the procedure described by Lichtenthaler [7]. Acetone (200 mL, technical grade) was added, followed by slow addition of liquid nitrogen (*ca.* 2 L). After complete addition of liquid nitrogen, the Soxhlet thimble was transferred into a Soxhlet extractor and warmed to 10–20 °C. One part of acetone (300 mL) was added to the extractor and another part (1.5 L) was added into a 2 L separating flask connected to the extractor. The solvent was refluxed under an Ar atmosphere for *ca.* 24 h. During this time, 70 to 75 extraction cycles yielded a deep green chlorophyll extract. After extraction was completed, the color of the extraction thimble had changed from deep green to light yellow. The acetone extract was filtered through cotton wool to remove traces of biomass and the solvent was removed in a rotavapor. The water-containing residue was dissolved in CH_2Cl_2 (200 mL) and the water was removed by azeotropic distillation in a rotavapor. The residue was carefully dried *in vacuo* using an oil pump, suspended in dry MeOH (500 mL) and concentrated H_2SO_4 (25 mL) was cautiously added. The mixture was stirred for 20 h at room temperature under an Ar atmosphere with exclusion of light. To the mixture were added CH_2Cl_2 (400 mL), H_2O (800 mL), saturated aqueous solution of NaCl (200 mL) and a 10% aqueous solution of $NaHCO_3$ (300 mL). The organic layer was separated and the aqueous layer extracted three times with CH_2Cl_2 (200 mL portions). The combined organic extracts were washed twice with water (500 mL portions), dried by filtration through cotton wool and evaporated in a rotavapor. The red crude extract was carefully dried *in vacuo* using an oil pump, dissolved in a small amount of CH_2Cl_2 (maximum 40 mL) and subjected to column chromatography (300 g silica gel, 32–63 µm, 60 Å). (Alternatively, column chromatography with Alox [400 g Alox N, activity II–III; eluent: *n*-hexane to *n*-hexane/CH_2Cl_2 (1:1) to CH_2Cl_2/acetone (15:1)] can be applied.) Elution with *n*-hexane gave a yellow/red fraction of carotenoids. More of a yellow/red fraction was eluted with *n*-hexane/CH_2Cl_2 (1:1). After elution with CH_2Cl_2 and then with CH_2Cl_2/acetone (30:1) the green main fraction of methyl pheophorbide *a* was obtained. A final fraction eluted with MeOH was treated with H_2SO_4/MeOH and it yielded after work up and chromatography a minor amount of methyl pheophorbide *a*. The combined methyl pheophorbide *a* fractions were crystallized from CH_2Cl_2/MeOH (*ca.* 1.5:10) to give dark green crystals of methyl pheophorbide *a* **2** as a 89:11 mixture of 13^2R: 13^2S diastereomers. Yield 4.2 g (0.6% related to biomass), mp 226–228 °C. lit. 206 °C [3a], 228 °C [14]. TLC (cellulose, *n*-heptane/pyridine, 7:3) R_f = 0.75; (silica gel, CCl_4/acetone, 5:1) R_f = 0.41. HPLC [LiChrosorb Si 60 Å (Merck Milipore), CH_2Cl_2/EtOAc, 92.5:7.5, 1.5 mL/min, UV detect. at 405 nm] R_t (13^2S) = 7.6 min, R_t (13^2R) = 8.4 min. UV-vis (THF): $\lambda_{max,}$ nm (ε) 274 (15700), 322 (19700), 411 (106000), 470 (4300), 505 (11800), 535 (10600), 560 (3060), 609 (8250), 668 (51100). IR (KBr): ν, cm^{-1} 2960 (m, CH), 1732 (s, CO ester), 1700 (s, CO keto), 1621 (m), 1498 (m), 1297 (m), 1203 (m), 1164 (s), 1035 (m), 991 (m). MS (EI, 70 eV, 200 °C): *m/z* (%) 606 (100) [M^+], 548 (29) 459 (17), 236 (10). MS (ESI, positive, CH_2Cl_2/MeOH, 1:10): *m/z* 607 $[M + H]^+$, 629 $[M + Na]^+$, 645 $[M + K]^+$. MS (ESI, negative, CH_2Cl_2/MeOH, 1:10): *m/z* 605 $[M–H]^-$.

***Methyl* 17*S*,18*S*-[8-*ethyl*-17-(2-*methoxycarbonylethyl*)-15-*methoxycarbonylmethyl*-2,7,12,18-*tetramethyl*-3-*vinyl*-17,18-*dihydro*-21H,23H-*porphyrin*-13-*yl]carboxylate* (*chlorin* e_6 *trimethylester*) (3).** Variant A: Methyl pheophorbide a **2** (1.7 g, 2.8 mmol was dissolved under an Ar atmosphere in degassed dry THF (20 mL) and $CHCl_3$ (100 mL) was added followed by 1 M KOH in MeOH (10 mL). The mixture was stirred with exclusion of light for 30 min at room temperature. The reaction mixture was poured into ice water (400 mL) and extracted twice with diethyl ether (100 mL portions). The combined organic layers were washed four times with water (200 mL portions) and dried by filtration through warm cotton wool. The solvent was evaporated in a rotavapor and the residue purified by column chromatography [200 g silica gel, 32–63 µm, 60 Å, CH_2Cl_2/petroleum ether/acetone (10:10:1)]. After removal of the eluent, the residue was crystallized from acetone/MeOH to give black-blue crystals of chlorin e_6 trimethylester **3**. Yield 1.3 g (73%), mp (acetone/MeOH) 210 °C, lit. 211 °C [15].

Variant B: Methyl pheophorbide *a* **2** (151.6 mg, 0.25 mmol) was dissolved under an Ar atmosphere in THF (10 mL) and then MeOH (100 mL) was added followed by $KOCH_3$ in MeOH (2 mL 35% solution). The mixture was stirred with exclusion of light for 30 min at room temperature. The reaction was monitored by TLC. After the reaction was complete, the mixture was poured into water (400 mL) and extracted twice with diethyl

ether (100 mL portions). The combined organic extracts were washed four times with water (200 mL portions) and dried by filtration through cotton wool. The solvent was removed in a rotavapor and the crude product purified by column chromatography [40 g silica gel, 32–63 µm, 60 Å, CH_2Cl_2/EtOAc (7:1). After removal of the eluent, crystallization of the fraction from acetone/MeOH gave black-blue crystals of chlorin e_6 trimethylester **3**. Yield 135 mg (85%), mp (acetone/MeOH) 207–208 °C. TLC (silica gel, CH_2Cl_2/EtOAc, 7:1): R_f = 0.59. HPLC [LiChrosorb Si 60 Å (Merck Milipore), CH_2Cl_2/EtOAc, 92.5:7.5, 1 mL/min, UV detect. at 405 nm]: R_t = 5.85 min. UV-vis (MeOH): λ_{max}, nm (ε) 303 (9540), 400 (162620), 500 (20510), 605 (11570), 660 (51100). UV-vis (THF): λ_{max}, nm (ε) 303 (9540), 401 (123500), 500 (11260), 529 (4680), 558 (1690), 609 (4520), 665 (43600). IR (KBr): ν, cm^{-1} 3296 (w, NH), 2950 (m, CH), 2914 (w), 1731 (s, CO ester), 1601 (m), 1440 (m), 1240 (m), 1165 (m), 1064 (m). ^{1}H-NMR (200 MHz, $CDCl_3$): δ, ppm -1.42 (m, br, 21-NH, 23-NH), 1.61, 1.72, 1.77, 1.81 (m, 7H, 8^2-CH_3, 18-CH_3, 17^1-CH), 2.16–2.61 (m, 3H, 17^1-CH, 17^2-CH_2), 3.32 (s, 3H, 7-CH_3), 3.48 (s, 3H, 2-CH_3), 3.59 (s, 3H, 12-CH_3), 3.65 (s, 3H, 17-$COOCH_3$), 3.79 (s, 3H, 15-$COOCH_3$), 4.26 (s, 3H, 13-$COOCH_3$), 4.4–4.49 (t, 2H, 17-CH, 18-CH), 5.19–5.43 (q, 2H, 15^1-CH_2), 6.15–6.41 (q, 2H, 3^2 = CH_2), 8.0–8.15 (q, 1H, 3^1-CH), 8.8 (s, 1H, 20-CH), 9.6 (s,1H, 5-CH), 9.75 (s, 1H, 10-CH). MS (EI, 70 eV, 200 °C): *m/z* (%) 638 (100) $[M]^+$, 579 (13) $[M\text{-}COOCH_3]^+$, 565 (26) $[M - CH_2COOCH_3]^+$, 479 (20), 289 (7), 236 (7). MS (DCI, negative, NH_3, 8 mA/sec): *m/z* (%) 641 (3) 640 (15), 639 (52), 638 (100) $[M]^-$. MS (ESI, positive, CH_2Cl_2/MeOH, 1:10): *m/z* 639 $[M + H]^+$. MS (ESI, negative, CH_2Cl_2/MeOH): *m/z* 637 $[M–H]^-$.

17*S*,18*S*-[8-*Ethyl*-17-(2-*carboxy-ethyl*)-15-*carboxy-methyl*-2,7,12,18-*tetramethyl*-3-*vinyl*-17,18-*dihydro*-21*H*,23*H*-*porphyrin*-13-*yl]carboxylate* (*chlorin* e_6) (4). Chlorin e_6 trimethylester **3** (147 mg, 0.23 mmol) was dissolved under an Ar atmosphere in degassed acetone (10 mL) and degassed 15% aqueous KOH (10 mL) was added. The mixture was stirred at 40 °C for 40 min under an Ar atmosphere with exclusion of light and then refluxed for 2.5 h. After cooling to room temperature, the mixture was poured into water (90 mL) and 2 M HCl (12 mL) and stored overnight in a refrigerator. The formed micro-crystalline precipitate was separated by centrifugation. The precipitate was suspended three times in water (10 mL portions) and centrifugated. Water was completely removed by freeze drying and a dark green powder of quite pure chlorin e_6 **4** was obtained. Yield 125 mg (90%), mp > 230 °C. For further transformation the purity of **4** is sufficient. To achieve chlorin e_6 of higher purity, a tedious MPL chromatography is required [Silica gel RP-8 (Merck#11447, 40–63 µm, L 240 mm x diameter 10 mm), MeOH/CH_2Cl_2/H_2O (4:3:1), 2 mL/min]. HPLC [LiChrosorb 100 RP-18 (Merck Milipore), MeOH, $Bu_4NH_2PO_4$ (2.5 mmol/L) H_2O, 95:5, 1 mL/min, UV detect. at 405 nm]: R_t = 4.8 min. UV-vis (MeOH) λ_{max}, nm (ε) 286 (14320), 401 (139600), 502 (11450), 607 (4294), 661 (40380). ^{1}H-NMR (600 MHz, $CDCl_3$/pyridine-D_5) δ, ppm -0.9, -1.8 (2s, 2H, 21-NH, 23-NH), 1.73 (m, 4H, 8^2-CH_3, 17^1-CH), 2.28 (m, 2H, 17^2-CH_2), 2.67 (m, 1H, 17^1-CH_2), 3.29 (s, 3H, 7-CH_3), 3.50 (s, 3H, 2-CH_3), 3.63 (s, 3H, 12-CH_3), 3.8 (q, J = 7.2 Hz, 2H, 8^1-CH_2), 4.62 (dd, 2H, 17-CH, 18-CH), 5.5 (q, 2H, J = 7.8 Hz, 15^1-CH_2), 6.15, 6.17 (dd, 2H, 3^2 = CH_2), 8.19, 8.21, 8.24, 8.25 (dd, 1H, 3^1-CH), 9.05 (s 1H, 20-CH), 9.67 (s, 1H, 5-CH), 9.8 (s, 1H, 10-CH). MS (ESI, positive, MeOH): m/z 597 $[M + H]^+$. MS (ESI, negative, MeOH): m/z 595 $[M–H]^-$.

***Methyl* 17*S*,18*S*-[8-*ethyl*-17-(2-*carboxy-ethyl*)-15-*carboxymethyl*-2,7,12,18-*tetramethyl*-3-*vinyl*-17,18-*dihydro*-21*H*,23*H*-*porphyrin*-13-*yl*]*carboxylate* (*chlorin* e_6 *monomethylester*) (5).** Chlorin e_6 trimethylester **3** (200 mg, 0.31 mmol) was suspended under an Ar atmosphere in 5 M aqueous HCl (50 mL) and heated to 90 °C for 30 min. After cooling to room temperature, ice water (200 mL) was added to the mixture and it was exhaustively extracted with EtOAc (total 1.5 L). The combined organic extracts were washed three times with water (200 mL portions), dried by filtration through cotton wool and evaporated in a rotavapor. The crude product was isothermally crystallized from $CHCl_3$/petroleum ether to give chlorin e_6 monomethylester **5** as dark violet crystals. Yield 155 mg (82%), mp ($CHCl_3$/petroleum ether) > 230 °C. UV-vis (DMSO): λ_{max}, nm (ε) = 403 (113250), 501 (10200), 530 (4170), 560 (2160), 608 (4090), 663 (26900). IR (KBr): ν, cm^{-1} 3420 (m), 2960 (m, C-H), 2920 (m, C-H), 2860 (m, C-H), 1720 (s, CO), 1600 (s, CO), 1440 (m), 1380 (m), 1255 (m), 1215 (m), 1065 (m), 800 (w), 730 (w), 670 (w). ^{1}H-NMR (600 MHz, $CDCl_3$/pyridine-D_5): δ, ppm 1.53 (t, ^3J = 7.2 Hz, 3H, 8^2-CH_3), 1.61 (d, ^2J = 8.0 Hz, 3H, 18-CH_3), 1.67, 2.21 (2s, 2H, 17^1-CH_2), 2.16, 2.50 (m, 2H, 17^2-$COCH_2$), 3.10 (s, 3H, 7-CH_3), 3.26 (s, 3H, 2-CH_3), 3.39 (s, 3H, 12-CH_3), 3.59 (q, ^2J = 7.2 Hz, 2H, 8^1-CH_2), 4.07 (s, 3H, 13-$COOCH_3$), 4.36 (q, ^3J = 8.0 Hz, 1H, 18-CH), 4.50 (d, 1H, 17-CH), 5.24, 5.31 (q, ^3J = 7.8 Hz, 2H, 15^1-CH_2), 5.94, 6.16 (dd, 2H, 3^2 = CH_2), 7.89 (dd, 1H, 3^1-CH), 8.62 (s, 1H, 20-CH), 9.41 (s, 1H, 5-CH), 9.51 (s, 1H, 10-CH). ^{13}C-NMR (90 MHz, $CDCl_3$/pyridine-D_5): δ, ppm 10.5 (7-*C*H_3), 11.4 (2-*C*H_3), 11.5 (12-*C*H_3), 16.8 (8-*C*H_3), 18.9 (8^1-*C*H_2), 22.5 (18-*C*H_3), 29.4 (17^1-*C*H_2), 31.1 (17^2-CO*C*H_2), 38.4 (15^1-*C*H_2), 48.8 (18-*C*H), 52.3 (13-O*C*H_3), 52.5 (17-*C*H), 93.2 (20-*C*H), 97.8 (5-*C*H), 101.1 (10-*C*H), 103.2 (15-*C*), 121.0 (3^2-*C*H_2), 123.3 (13-*C*), 128.9 (11-N=*C*), 129.0 (3^1-*C*H), 129.8 (2-*C*), 133.9 (3-*C*), 134.4 (4-N=*C*), 135.2 (14-N=*C*), 135.3 (7-*C*), 135.8 (12-*C*), 138.5 (1-N=*C*), 144.5 (8-*C*), 148.2 (9-N=*C*), 153.9 (6-N=*C*), 167.6 (16-N=*C*), 169.2 (13-*C*=O), 169.2 (19-N=*C*), 174.7 (15-*C*OOH), 175.4 (17-*C*OOH). MS (ESI, positive, CH_2Cl_2/MeOH, 1:10): *m/z* 611 $[M + H]^+$.

17*S*,18*S*-{8-*Ethyl*-17-[2-(*glucitol*-1-*yl-carbamoyl*)-*ethyl*]-15-(*glucitol*-1-*yl-carbamoyl*)-*methyl*-2,7,12,18-*tetramethyl*-3-*vinyl*-17,18-*dihydro*-21*H*,23*H*-*porphyrin*-13-*yl*}-*carboxylate* (*chlorin* e_6 *glucodiamide*) (6). Chlorin

e_6 **4** (45 mg, 0.075 mmol) was dissolved in dry THF (20 mL) and NEt_3 (0.36 mL) was added. The solution was cooled to -15 °C and i-BuOCOCl (0.3 mL, 2.3 mmol) in dry THF (5 mL) was slowly added. The mixture was stirred at -15 °C for 15 min until the anhydride had formed completely. Glucamine (150 mg, 0.83 mmol) in EtOH (1.6 mL), NEt_3 (1.0 mL) and H_2O (1.0 mL) were added dropwise and the mixture was stirred at room temperature for 15 h. The mixture was concentrated in a rotavapor and the residue dissolved in H_2O (*ca.* 10 mL). After washing the aqueous phase with EtOAc (*ca.* 10 mL), water was removed by lyophilization. The residue was purified by reversed phase column chromatography [RP-18 SiO_2 (Fluka), MeOH/H_2O (4:3) — MeOH]. After removal of the eluent in a rotavapor and *in vacuo* using an oil pump, chlorin e_6 glucodiamide **6** was obtained as a green powder. Yield 61 mg (88%), mp. > 230. UV/vis (MeOH): λ_{max}, nm (ε) = 400 (102000), 499 (9200), 530 (5500), 607 (4800), 662 (29300). IR (KBr): ν, cm^{-1} 3410 (s, br, OH, NH), 2965 (m, C-H), 2935 (m, CH), 2850 (m, C-H), 1690 (m, C=O carboxyl), 1620 (s, C=O amide), 1540 (m, C=C), 1470 (m, CH), 1430 (m), 1265 (m, C-O), 1160 (w), 1080 (m), 1030 (w), 725 (m). MS (ESI, positive, MeOH): m/z 872 $[M + H]^+$, 899 $[M + Na]^+$, 915 $[M + K]^+$; (ESI, negative, MeOH): m/z 911 $[M + Cl]^-$.

***Methyl* 17*S*,18*S*-{8-*ethyl*-17-[2-(*glucitol*-1-*yl*-*carbamoyl*)-*ethyl*]-15-(*glucitol*-1-*yl*-*carbamoyl*)-*methyl*-2,7,12,18-*tetramethyl*-3-*vinyl*-17,18-*dihydro*-21*H*,23*H*-*porphyrin*-13-*yl*}*carboxylate* (*chlorin e_6 monomethylester glucodiamide*) (7).** Chlorin e_6 monomethylester **5** (20 mg, 0.033mmol) was dissolved in dry THF (5 mL) and NEt_3 (0.12 mL) was added. The solution was cooled to -15 °C and i-BuOCOCl (0.2 mL, 1.5 mmol) in dry THF (5 mL) was slowly added. The mixture was stirred at -15 °C for 1 h until the anhydride had formed completely. Glucamine (60 mg, 0.33 mmol) in EtOH (0.5 mL) and H_2O (0.5 mL), NEt_3 (0.5 mL) and H_2O (0.5 mL) were added dropwise and the mixture was stirred at room temperature for 15 h. The mixture was concentrated in a rotavapor and the residue was dissolved in H_2O (*ca.* 10 mL). After washing the aqueous phase with EtOAc (*ca.* 10 mL), water was removed by lyophilization. The residue was purified by reversed phase column chromatography [RP-18 SiO_2 (Fluka), MeOH/H_2O (1:1) – MeOH/H_2O (2:1) – MeOH]. After removal of the eluent in a rotavapor and *in vacuo* using an oil pump, chlorin e_6 monomethylester glucodiamide **7** was obtained as a green powder. Yield 18 mg (59%), mp. 132–133 °C. UV/vis (MeOH): λ_{max}, nm (ε) = 401 nm (102000), 500 (9300), 531 (5800), 606 (5100), 663 (28000). IR (KBr): ν, cm^{-1} 3395 (s, br, O-H, N-H), 2945 (m, C-H), 2910 (m, C-H), 2850 (m, C-H), 1710 (m, C=O ester), 1670 (s, C=C), 1540 (m, C=C), 1440 (C-H), 1385 (m), 1240 (m, C-O), 1070 (s), 725 (m). ^{1}H-NMR (600 MHz, DMSO-D_6): δ, ppm -1.58/-1.81 (2s, 2H, 21-NH, 23-NH), 1.58/2.11 (t, 2H, 17^1-CH_2), 1.67 (m, 6H, 18-CH_3, 8^2-CH_3), 2.08/2.42 (m, 2H, 17^2-CH_2), 3.02/3.24 (m, 2H, 15^4-NCH_2), 3.09/3.40 (m, 2H, 17^5-NCH_2), 3.30 (s, 3H, 7-CH_3), 3.35/3.53 (m, 4H, 15^9-OCH_2, 17^{10}-OCH_2), 3.40–3.70 (m, 8H, 15^5–15^8-OCH, 17^5–17^9-OCH), 3.52 (s, 3H, 2-CH_3), 3.54 (s, 3H, 12-CH_3), 3.81 (q, 2H, 8^1-CH_2), 4.19 (s, 3H, 13-OCH_3), 4.20–4.70 (m, 10H, 15^5-15^9-OH, 17^6–17^{10}-OH), 4.35 (d, 1H, 17-CH), 4.57 (m, 1H, 18-CH), 5.08 (s, 2H, 15^1-CH_2), 6.19/6.46 (2d, 2H, 3^2 = CH_2), 7.71 (t, 1H, 15^3-NH), 7.79 (t, 1H, 17^4-NH), 8.31 (dd, 1H, 3^1-CH), 9.08 (s, 1H, 20-CH), 9.69 (s, 1H, 5-CH), 9.77 (s, 1H, 10-CH). ^{13}C-NMR (90 MHz, DMSO-D_6): δ, ppm 10.8 (7-CH_3), 11.7 (12-CH_3), 11.9 (2-CH_3), 17.6 (8^2-CH_3), 18.6 (8^1-CH_2), 22.8 (18-CH_3), 30.4 (17^1-CH_2), 32.5 (17^2-CH_2), 39.0 (15^1-CH_2), 41.8 (15^4-NCH_2), 42.4 (17^5-NCH_2), 48.3 (18-CH), 52.7 (17-CH), 53.1 (13-OCH_3), 63.1 (15^{10}-OCH_2, 17^{10}-OCH_2), 69.4–74.4 (15^5–15^8-OCH, 17^6-17^9-OCH), 94.1 (20-CH), 98.2 (5-CH), 101.4 (10-CH), 122.2 (3^2 = CH_2), 129.3 (3^1 = CH). MS (ESI, positive, MeOH): m/z 959 $[M + Na]^+$, 975 $[M + K]^+$. (ESI, negative, MeOH): m/z 971 $[M + Cl]^-$.

***Methyl* 17*S*,18*S*-{8-*ethyl*-17-[2-(*arabitol*-1-*yl*-*carbamoyl*)-*ethyl*]-15-(*arabitol*-1-*yl*-*carbamoyl*)-*methyl*-2,7,12,18-*tetramethyl*-3-*vinyl*-17,18-*dihydro*-21*H*,23*H*-*porphyrin*-13-*yl*}-*carboxylate* (*chlorin e_6 monomethylseter arabodiamide*) (8).** Chlorin e_6 monomethylester **5** (39 mg, 0.063 mmol) was dissolved in dry THF (10 mL) and NEt_3 (0.35 mL) was added. The solution was cooled to -15 °C and i-BuOCOCl (0.25 mL, 1.9 mmol) in dry THF (5 mL) was slowly added. The mixture was stirred at -15 °C for 30 min until the anhydride had formed completely. L-1-amino-1-deoxy-arabitinol [10] (200 mg, 1.9 mmol) in EtOH (0.8 mL), NEt_3 (0.5 mL) and H_2O (0.6 mL) were added dropwise and the mixture was stirred at room temperature for 15 h. The mixture was concentrated in a rotavapor and the residue dissolved in H_2O (*ca.* 10 mL). After washing the aqueous phase with EtOAc (*ca.* 10 mL), water was removed by lyophilization. The residue was purified by reversed phase column chromatography [RP-18 SiO_2 (Fluka), MeOH/H_2O (1:1) – MeOH/H_2O (2:1) – MeOH]. After removal of the eluent in a rotavapor and *in vacuo* using an oil pump, chlorin e_6 monomethylester arabodiamide **8** was obtained as a green powder. Yield 34 mg (61%), mp. > 230 °C. UV/vis (MeOH): λ_{max}, nm (ε) 400 (103000), 501 (9400), 532 (5700), 607 (5000), 663 (29000). IR (KBr): ν, cm^{-1} 3395 (s, br, OH, NH), 2945 (m, CH), 2910 (m, CH), 2850 (m, CH), 1710 (m, C=O ester), 1600 (s, C=O amide), 1540 (m, C=C), 1440 (m, C-H), 1385 (m), 1240 (m, CO), 1070 (s), 725 (m). ^{1}H-NMR (200 MHz, DMSO-D_6): δ, ppm -1.59/-1.83 (2s, 2H, 21-NH, 23-NH), 1.65/2.14 (t, 2H, 17^1-CH_2), 1.65 (m, 6H, 18-CH_3, 8^2-CH_3), 2.84 (t, 2H, 17^2-CH_2), 3.03 (m, 2H, 15^4-NCH_2), 3.16 (m, 2H, 17^5-NCH_2), 3.19 (s, 3H, 7-CH_3), 3.47 (m, 4H, 15^8-OCH_2, 17^9-O-CH_2), 3.40–3.60 (m, 6H, 15^5–15^7-OCH, 17^5–17^8-OCH), 3.49 (s, 3H, 2-CH_3), 3.52 (s, 3H, 12-CH_3), 3.75 (q, 2H, 8^1-CH_2), 4.09 (s, 3H, 13-OCH_3), 4.20–4.50 (m, 8 H, 15^5–15^8-OH, 17^6–17^9-OH), 4.35 (d, 1H, 17-CH), 4.45 (d, 1H, 18-CH), 5.02 (m, 2H, 15^1-CH_2), 6.20/6.49 (2d, 2H, 3^2 = CH_2), 7.77 (t, 1H, 15^3-NH), 7.83 (t, 1H, 17^4-NH), 8.31 (dd, 1H, 3^2 = CH), 9.06 (s, 1H, 20-CH), 9.69 (s, 1H, 5-CH),

9.75 (s, 1H, 10-CH). MS (ESI, positive, MeOH): m/z 872 $[M + H]^+$, 899 $[M + H]^+$, 915 $[M + K]^+$; (ESI, negative, MeOH): m/z 911 $[M + Cl]^-$.

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Supporting information

Comprehensive NMR data for **2**, **3**, **4**, **5** and **7** are given in the supplementary material. This material is available free of charge *via* the Internet htpp://www.worldscinet.com/jpp/jpp.shtml.

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