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Syntheses and Cellular Investigations of 17^3-, 15^2-, and 13^1-Amino Acid Derivatives of Chlorin e_6

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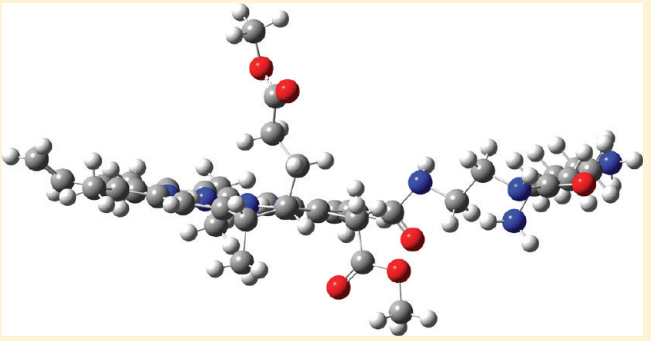
Supporting Information

ABSTRACT: A series of amino acid conjugates of chlorin e_6, containing lysine or aspartic acid residues in positions 17^3, 15^2, or 13^1 of the macrocycle were synthesized and investigated as photosensitizers for photodynamic therapy of tumors. All three regioisomers were synthesized in good yields and in five steps or less from pheophytin *a* (**1**). In vitro investigations using human carcinoma HEp2 cells show that the 15^2-lysyl regioisomers accumulate the most within cells, and the most phototoxic are the 13^1 regioisomers. The main determinant of biological efficacy appears to be the conjugation site, probably because of molecular conformation. Molecular modeling investigations reveal that the 17^3-substituted chlorin e_6 conjugates are L-shaped, the 15^2 and 13^1 regioisomers assume extended conformations, and the 13^1 derivatives are nearly linear. It is hypothesized that the 13^1-aspartylchlorin e_6 conjugate may be a more efficient photosensitizer for PDT than the commercial currently used 15^2 derivative.



INTRODUCTION

Photodynamic therapy (PDT) is a binary cancer therapy that relies on the selective uptake of a photosensitizer into cancer cells followed by irradiation with red light, which produces singlet oxygen and other reactive oxygen species.[1−4] Highly cytotoxic singlet oxygen readily reacts with electron-rich biomolecules in its vicinity such as unsaturated lipids, amino acids, and DNA.[5] Because of the known limited diffusion of singlet oxygen through tissues the PDT effects are largely localized to the photosensitizer-containing cells, thus reducing potential damage to normal cells in the vicinity of the tumor. The selectivity of the PDT treatment depends both on the tumor-targeting ability of the photosensitizer and the light used to activate it. An ideal photosensitizer should have minimal toxicity in the dark, a high quantum yield of triplet state formation in the presence of light, high selectivity for tumor cells over normal cells, rapid clearance from normal tissues, and a strong absorption peak within the "therapeutic window" (600−800 nm) for optimal light penetration through tissue.

Commercial hematoporphyrin derivative is a porphyrin-based photosensitizer that has been commercially developed and approved in several countries for the PDT treatment of melanoma, early and advanced stage cancer of the lung, digestive tract, genitourinary tract, and Barrett's esophagus.[3−6] However, it has only a weak absorption within the therapeutic window and often induces skin photosensitivity in patients. For these reasons, several second-generation photosensitizers bearing stronger and red-shifted absorption within the therapeutic window have been investigated in PDT; these include tetra(*meta*-hydroxyphenyl)chlorin, (mTHPC), 2-[1-hexyloxyethyl]-2-devinyl-pyropheophorbide *a* (HPPH), lutetium(III) texaphyrin (LuTex), mono-L-aspartylchlorin e_6, and phthalocyanine 4 (Pc4).[3−6] Chlorins (dihydroporphyrins) are particularly promising photosensitizers for PDT because of their intense absorptions above 640 nm; in particular, chlorophyll *a* derivatives are inherently amphiphilic macrocycles that have been extensively investigated and shown to have low dark toxicities while being able to effectively generate singlet oxygen upon light activation.[7−9] Among these, HPPH and mono-L-aspartylchlorin e_6 have been investigated and are currently in advanced-stage clinical trials for oncologic PDT applications;[10−13] both of these chlorophyll *a* based compounds have shown superior PDT activity as well as rapid clearance from normal tissue and decreased patient photosensitivity compared with commercial hematoporphyrin derivative.

Chlorophyll *a* derivatives of the chlorin e_6 series possess three carboxylic side chains that can be derivatized in multiple ways to produce novel amphiphilic photosensitizers for PDT investigations. For example, their conjugation to peptides, sugars, lipoproteins, and polyamines have been reported.[14−19] In particular, amino acid residues have been found to improve the biological effects of porphyrin-based compounds, and their nature and position about the macrocycle can have a significant impact on PDT efficacy.[5,20,21] In the present study, we synthesized and investigated 17^3, 15^2, and 13^1 amino acid conjugates of chlorin e_6 (Figure 1) to evaluate the effect of the nature, conjugation site, and position of the amino acid on the PDT efficacy of the conjugates in vitro, using human carcinoma HEp2 cells. The

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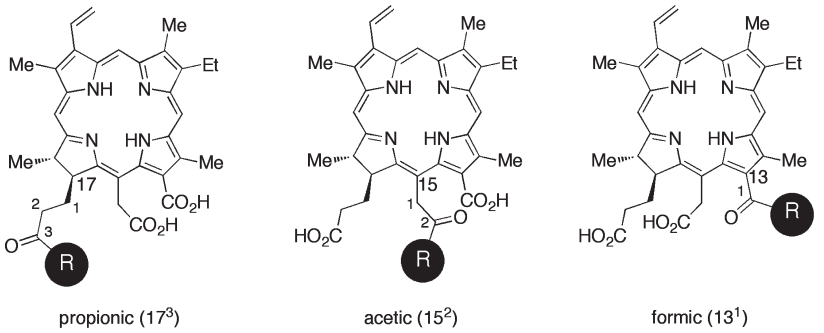
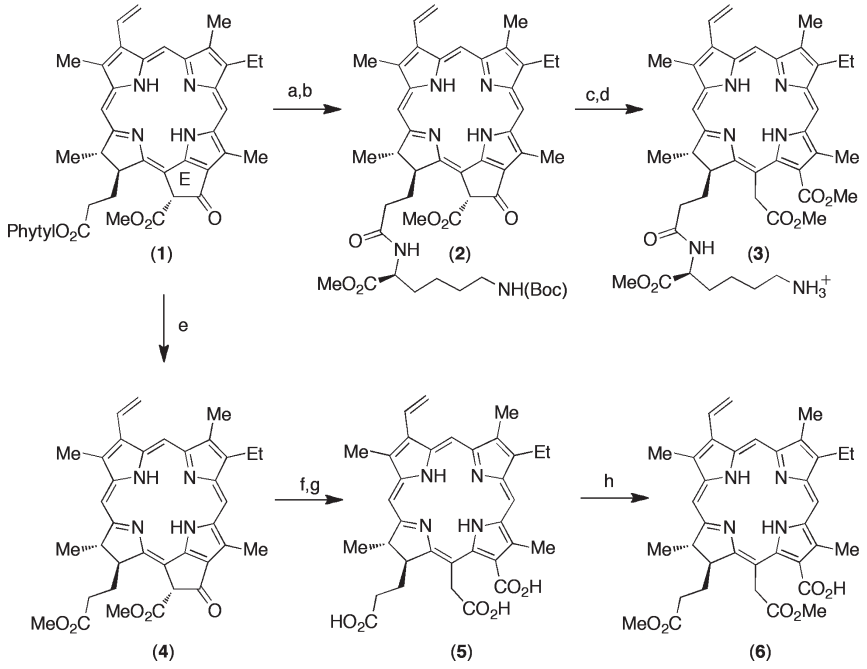


Figure 1. 17^3-, 15^2-, and 13^1-regioisomers of "R"-substituted chlorin e_6.

Scheme 1[a]



[a] Reaction conditions: (a) TFA:H_2O 4:1, 0 °C, 1 h, 90%; (b) DCC, DMAP, H-Lys(Boc)-OMe·HCl, DIEA, CH_2CL_2, rt, 14 h, 52%; (c) NaOMe, THF, 0 °C, 4 h, 89%; (d) TFA, CH_2Cl_2, thioanisole, 0 °C to rt, 12 h, 72%; (e) 5% H_2SO_4/MeOH, rt, 12 h, 96%; (f) NaOMe, THF, 0 °C, 4 h, 98%; (g) 18 equiv LiI, EtOH, reflux, 24 h, 21%; (h) 5% H_2SO_4/MeOH, rt, 12 h, 99%.

structure of mono-L-aspartylchlorin e_6, bearing the aspartyl residue in position 15^2 (rather than in 17^3 as initially proposed) has recently been established.[22] This study led us to design new syntheses of the 17^3 and 13^1 aspartyl regioisomers of mono-L-aspartylchlorin e_6 as well as to investigate the corresponding cationic lysine derivatives, which could potentially show stronger interactions with negatively charged biological molecules and plasma membranes and consequently enhanced PDT efficacy.[23] Furthermore, we investigated the introduction of a spacer group, ethylene diamine, and of metalation, via insertion of palladium(II). The photosensitizing properties of porphyrin derivatives are known to be modulated by inner coordinated metal ions and associated axial ligands.[24−26] In particular, Pd(II) complexes have been shown to be efficient generators of singlet oxygen, and the palladium(II)-bacteriopheophorbide is currently being evaluated in clinical trials for the treatment of prostate cancer.[27,28] The work described herein indicates that the site of amino acid conjugation, rather than the presence of Pd(II) and even the nature of amino acid, is the major determinant of phototoxicity.

RESULTS AND DISCUSSION

1. Syntheses. The synthetic route to the 17^3-lysyl conjugate of chlorin e_6 (**3**) is shown in Scheme 1. Pheophytin *a* (**1**) was obtained by extraction from *Spirulina pacifica* alga, an ideal source for chlorophyll *a* because of the complete absence of chlorophyll *b*. The selective hydrolysis of the phytyl ester group of pheophytin *a* (**1**) using aqueous TFA produced pheophorbide *a* in high yield without affecting the *β*-keto ester of the isocyclic ring.[29,30] This ring serves as a natural protecting group for the 13^1- and 15^2-positions during the coupling reactions. Selective hydrolysis of the phytyl ester produced a free 17^3-carboxylic acid group, which was activated with DCC/DMAP and coupled with H-lysine(OtBu) methyl ester to form the lysine(OtBu) methyl

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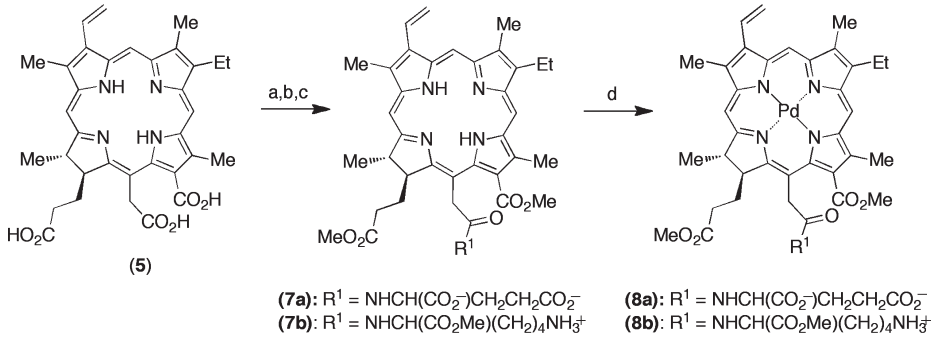
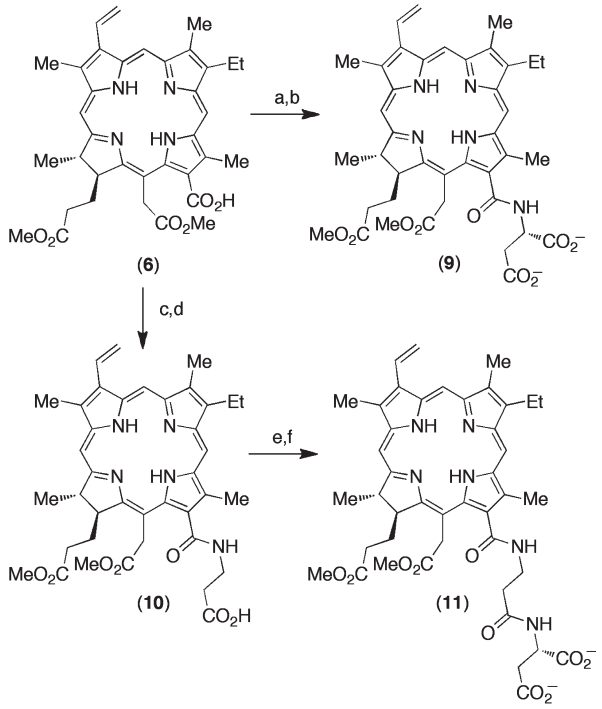
Scheme 2[a]



[a] Reaction conditions: (a) DCC, DMAP, CH_2Cl_2, rt, 2 h; H-Asp(Boc)$_2$·HCl or H-Lys(Boc)-OMe·HCl, DIEA, CH_2Cl_2, rt, 14 h; (b) CH_2N_2, CH_2Cl_2, 30 min; (c) TFA, CH_2Cl_2, thioanisole, 0 °C to rt, 12 h (**7a**, 45%; **7b**, 77%); (d) Pd(OAc)$_2$, THF, 60 °C, 3 h (**8a**, 98%; **8b**, 99%).

Scheme 3[a]



[a] Reaction conditions: (a) L-Asp(OtBu)$_2$·HCl, DIEA, CH_2Cl_2, HOBt, TBTU, DMF, rt, 14 h, 66%; (b) TFA, CH_2Cl_2, thioanisole, 0 °C to rt, 12 h, 88%; (c) β-Ala(OtBu)·HCl, DIEA, CH_2Cl_2, HOBt, TBTU, DMF, rt, 12 h, 68%; (d) TFA, CH_2Cl_2, thioanisole, 0 °C to rt, 12 h, 95%; (e) L-Asp(OtBu)$_2$·HCl, DIEA, CH_2Cl_2, HOBt, TBTU, DMF, rt, 12 h, 87%; (f) TFA, CH_2Cl_2, thioanisole, 0 °C to rt, 12 h, 97%.

ester pheophorbide *a* (**2**) in 52% yield.[32] ^{1}H NMR spectroscopy demonstrated the existence of the isocyclic ring and new peaks for the lysine residue. Subsequent isocyclic ring-opening with sodium methoxide[31] in THF produced the 17^3-lysyl(Boc) chlorin e_6 TME in high yield (89%); deprotection of the amine group of the lysine side chain with TFA in CH_2Cl_2 followed and yielded the 17^3-lysylchlorin e_6 (**3**) in 30% overall yield from pheophytin *a* (**1**).

To synthesize the 15^2 and 13^1 amino acid derivatives of chlorin e_6, pheophytin *a* (**1**) was converted into methyl pheophorbide *a* (**4**) by transesterification of the ester group using sulfuric acid in methanol, as previously reported,[31] followed by isocyclic ring-opening, as shown in Scheme 1. An optimized yield of 98% of chlorin e_6 trimethyl ester was obtained when 1.1 equiv of freshly prepared sodium methoxide in THF were used in the ring-opening reaction. Chlorin e_6 (**5**) was obtained by hydrolysis of the three methyl esters using an excess of LiI in ethyl acetate under reflux conditions for 24 h.[33] Alternative hydrolysis under basic conditions using NaOH and LiOH gave lower yields of chlorin e_6. Under acidic conditions the 13^1-carboxylic acid of chlorin e_6 is deactivated,[34] allowing for the selective methylation of the 15^2-and 17^3-carboxylic acids with 5% H_2SO_4/MeOH to give chlorin e_6 dimethyl ester (**6**) in quantitative yield.[22] Chorin e_6 (**5**) was used as starting material for the preparation of the 15^2 amino acid derivatives of chlorin e_6 (as shown in Scheme 2), whereas the chlorin (**6**) and methyl pheophorbide *a* (**4**) were used to prepare the 13^1 amino acid derivatives of chlorin e_6 (as shown in Schemes 3 and 4).

We have previously shown[22] that the 15^2-carboxylic acid of chlorin e_6 is the most reactive, regardless of the coupling reagent employed. Therefore, upon activation of chlorin e_6 (**5**) with 1 equiv of DCC/DMAP, followed by addition of protected amino acid, reaction with diazomethane[35] and deprotection under acidic conditions, the 15^2-aspartyl (**7a**) and -lysyl (**7b**) derivatives of chlorin e_6 were obtained in overall 36% and 56% yields, respectively (Scheme 2). The use of excess DCC significantly reduced the yields obtained due to the formation of the 17^3,15^2-diamino acid conjugate as a side product.

Insertion of palladium into the chlorin ring was attempted before the deprotection step to simplify the purification process; however, low yields of the target metallochlorins were obtained. Therefore, palladium insertion was performed after deprotection, using either palladium(II) acetate or palladium(II) chloride. Quantitative yields for the metal insertion reaction were obtained using 1.2 equiv of palladium(II) acetate in THF at 40 °C to produce the corresponding Pd(II) complexes (**8**). As expected, significant blue-shifts in the UV−vis spectrum along with ^{1}H NMR and mass spectrometry confirmed the insertion of palladium. Heavy metal atoms in the core of the tetrapyrroles have been shown to facilitate intersystem crossing and increase the production of singlet oxygen due to the inner heavy atom effect.[36,37]

Two routes were used to synthesize the 13^1-amino acid conjugates: (1) Selective esterification of 17^3- and 15^2-carboxylic acids of chlorin e_6 to produce the monocarboxylic acid (**6**) followed by

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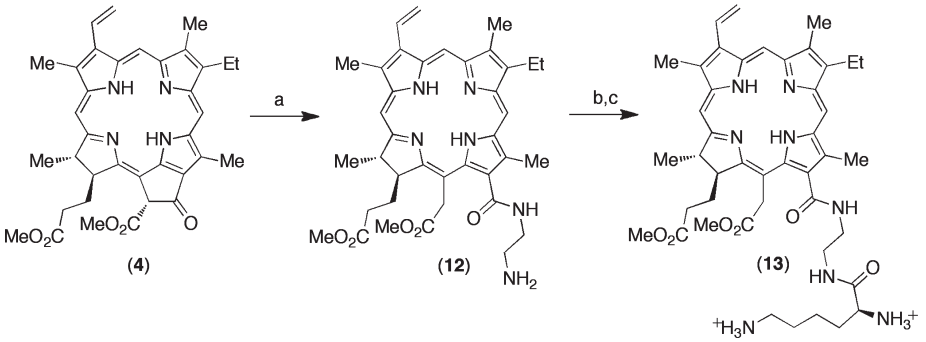
Scheme 4[a]



[a] Reaction conditions: (a) $NH_2C_2H_4NH_2$, $CHCl_3$, rt, 24 h, 91%; (b) (Boc)Lys(Boc)-OH, DIEA, CH_2Cl_2, HOBt, TBTU, DMF, rt, 12 h, 78%; (c) TFA, CH_2Cl_2, thioanisole, 0 °C to rt, 12 h, 81%.

coupling[34] (Scheme 3) and (2) isocyclic ring-opening of methyl pheoporbide *a* (**4**) with a nucleophile (Scheme 4). The 13^1-carboxylic acid of chlorin (**6**) was activated using HOBt/TBTU under basic conditions[38] and coupled with L-aspartic di(*tert*)butyl ester. With DCC/DMAP, a lower yield was obtained for the coupling reaction. Subsequent deprotection of both carboxylic acids in the aspartic chain provided (**9**) in 57% overall yield from chlorin e_6 (Scheme 3). Coupling of the 13^1-carboxylic acid of chlorin (**6**) with *β*-alanine *tert*-butyl ester followed by deprotection using TFA gave the 13^1-*β*-alanylchlorin e_6 dimethyl ester (**10**) in 64% overall yield. Aspartic di(*tert*)butyl ester was then coupled under similar conditions, followed by removal of the *tert*-butyl protecting group to give (**11**) in 54% yield from (**10**). *β*-Alanine provides a 4-atom link between the aspartic acid and chlorin e_6 dimethyl ester and allows for direct comparison with the lysine derivative **13** (see Scheme 4). The use of a linker significantly increased the yield of the coupling reaction (from 66 to 87%), and it can also affect the biological properties of the photosensitizer.[20,39]

The nucleophilic ring-opening of the isocyclic ring in pheophorbide *a* with ethylenediamine and ethanolamine has already been previously reported.[25,40] We took advantage of this high yielding isocyclic ring-opening reaction to develop a potentially high yielding synthetic route to novel chlorin e_6 photosensitizers directly from pheophytin *a* (**1**) in four steps. Nucleophilic ring-opening of the isocyclic ring with ethylenediamine produced molecule (**12**) in 91% yield. Subsequent coupling with protected lysine followed by deprotection gave the desired product (**13**) in four steps and in 55% overall yield from pheophytin *a* (**1**). No absorption quenching of the chlorin macrocycle was observed upon conjugation with the amino acid lysine in comparison with the aspartic acid derivative (see Figure S1 of the Supporting Information).

2. Molecular Modeling. Conformation analyses for 17^3-, 15^2-, and 13^1-lysylchlorin e_6 derivatives **3**, **7b**, and **13** were performed in both the gas phase and in water at the HF/6-31G level. These calculations were based on the atom coordinates from the X-ray structure of 15^2-aspartylchlorin e_6 tetramethyl ester.[22] The minimum energy conformations found for compounds **3**, **7b**, and **13** in the gas phase, shown in Figure 2a−c, respectively, were very similar to those found in water phase (Figure S2 of the Supporting Information). In addition, the conformation of a monocationic 13^1-lysylchlorin e_6 derivative without a linker was also investigated in the gas phase (Figure 2d). As expected, the lysine residue in the 17^3-lysyl derivative **3** is nearly perpendicular

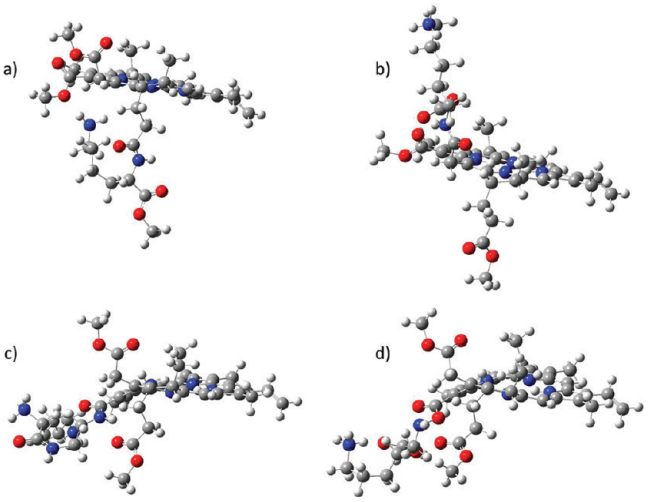


Figure 2. Energy minimized conformations in gas phase for chlorin e_6 derivatives (a) **3**, (b) **7b**, (c) **13**, and (d) $[13^1$ LysCe$_6$TME$]^+$. Optimization by energy was carried out at HF/6-31G level.

to the macrocyclic plane (Figure 2a), while in the 15^2-lysyl derivative **7b** it forms approximately a 120° angle (Figure 2b). On the other hand, the lysine residue extends away from the macrocycle in the 13^1-lysyl derivatives, with and without the short spacer (Figures 2c,d). Consequently, in the L-shape conformation of the 17^3-lysylchlorin e_6 derivative, the amino acid shelters one face of the chlorin ring, while in the case of the 15^2- and 13^1-lysyl derivatives, it extends away from the macrocycle, resulting in a nearly linear conformation for the 13^1 derivatives. The use of a short linker and the presence of two (rather than one) positive charges, as a result from conjugation to the C-terminus rather than the N-terminus of the amino acid, do not appear to have a significant effect upon the preferred conformation; the main determinant of molecule conformation is the site of substitution. While the minimum energy conformations found in water were similar to those in the gas phase, it is possible that in water intermolecular forces (such as $\pi-\pi$ stacking) predominate over the intramolecular interactions, in particular for the linear 13^1-lysylchlorin e_6 derivatives.

3. Cell Culture Studies. *3.1. Time-Dependent Cellular Uptake.* The results obtained for the time-dependent uptake of chlorin e_6 and its derivatives at a concentration of 10 μM in human HEp2

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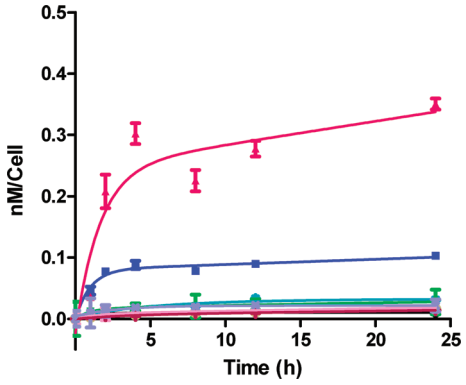

Figure 3. Time-dependent uptake of chlorin e_6 (**5**, black line) and its derivatives 17^3-LysChlorin e_6TME (**3**, brown line), 15^2-AspChlorin e_6DME (**7a**, light-blue line), 15^2-LysChlorin e_6TME (**7b**, red line), 15^2-AspPdChlorin e_6DME (**8a**, green line), 15^2-LysPdChlorin e_6TME (**8b**, blue line), 13^1-AspChlorin e_6DME (**9**, maroon line), 13^1-βAlaAspChlorin e_6DME (**11**, purple line), and 13^1-EDLysChlorin e_6DME (**13**, pink line) at 10 μM by HEp2 cells.

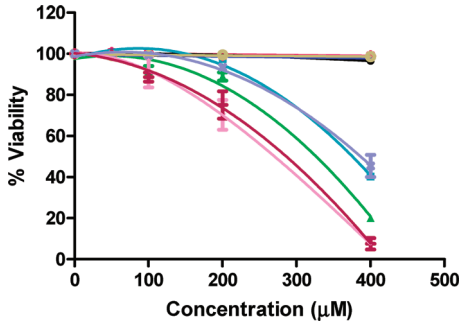

Figure 4. Dark toxicity of chlorin e_6 (**5**, black line) and its derivatives 17^3-LysChlorin e_6TME (**3**, brown line), 15^2-AspChlorin e_6DME (**7a**, light blue line), 15^2-LysChlorin e_6TME (**7b**, red line), 15^2-AspPdChlorin e_6DME (**8a**, green line), 15^2-LysPdChlorin e_6TME (**8b**, blue line), 13^1-AspChlorin e_6DME (**9**, maroon line), 13^1-βAlaAspChlorin e_6DME (**11**, purple line), and 13^1-EDLysChlorin e_6DME (**13**, pink line) toward HEp2 cells using 1 J/cm^2 light dose and the Cell Titer Blue assay.

cells are shown in Figure 3 (also see Figure S3 of the Supporting Information). All amino acid conjugates of chlorin e_6 were readily taken up by cells and showed uptake kinetics similar to those of unconjugated chlorin e_6. Interestingly, the 15^2-lysylchlorin e_6 derivatives **7b** and its palladium(II) complex **8b** accumulated to a much higher extent than all other compounds at all time points studied. In comparison with chlorin e_6, the lysyl derivatives **7b** and **8b** showed 18-fold and 4-fold higher cellular uptake, respectively, after 24 h. The observed high uptake for derivatives **7b** and **8b** is probably due to the lysine residue in position 15^2 because the corresponding aspartyl derivatives **7a** and **8a** accumulated to a significantly lower extent within cells. Presumably, the stronger interactions between the positively charged lysine derivatives (compared with the corresponding aspartyl derivatives) with the negatively charged plasma membrane leads to enhanced cellular uptake.[20,23] On the other hand, a lysine residue in position 17^3, as in derivative **3**, showed a dramatic decrease in cellular uptake compared with the same residue at position 15^2, suggesting that the molecule conformation plays an important role on the mechanism of cellular uptake. Indeed the

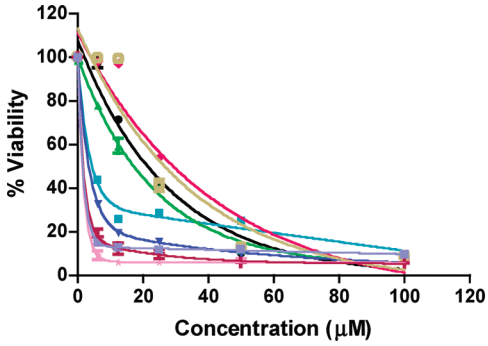

Figure 5. Phototoxicity of chlorin e_6 (**5**, black line) and its derivatives 17^3-LysChlorin e_6TME (**3**, brown line), 15^2-AspChlorin e_6DME (**7a**, light-blue line), 15^2-LysChlorin e_6TME (**7b**, red line), 15^2-AspPdChlorin e_6DME (**8a**, green line), 15^2-LysPdChlorin e_6TME (**8b**, blue line), 13^1-AspChlorin e_6DME (**9**, maroon line), 13^1-βAlaAspChlorin e_6DME (**11**, purple line), and 13^1-EDLysChlorin e_6DME (**13**, pink line) toward HEp2 cells using 1 J/cm^2 light dose and the Cell Titer Blue assay.

Table 1. Cytotoxicity (HEp2 cells) for Chlorin e_6 and Its Derivatives Using the Cell Titer Blue Assay

compd	dark toxicity (IC_{50}, μM)	phototoxicity (IC_{50}, μM)	ratio
chlorin e_6 (**5**)	>400	20.8	>19.2
17^3-LysChlorin e_6TME (**3**)	>400	26.2	>15.3
15^2-AspChlorin e_6DME (**7a**)	373.1	4.0	93.4
15^2-LysChlorin e_6TME (**7b**)	>400	28.8	>13.9
15^2-AspPdChlorin e_6DME (**8a**)	324.8	16.7	19.4
15^2-LysPdChlorin e_6TME (**8b**)	>400	3.3	>121.2
13^1-AspChlorin e_6DME (**9**)	284.6	0.61	466.6
13^1-βAlaAspChlorin e_6DME (**11**)	383.9	0.82	468.2
13^1-EDLysChlorin e_6DME (**13**)	268.4	1.34	200.3

extended conformation of the 15^2-lysylchlorin e_6 derivative **7b** (Figure 2b) might be the most favored for penetration across the plasma membrane, compared with the L-shape and linear conformations of the 17^3- and 13^1-lysyl derivatives (Figures 2a,c), respectively. The presence of a chelated palladium ion, as well as the more linear structure of the 13^1-lysyl substituent might lead to enhanced $\pi-\pi$ stacking of the macrocycles, thereby decreasing cellular uptake.

3.2. Cytotoxicity. The dark cytotoxicity and phototoxicity of chlorin e_6 and its derivatives was evaluated in HEp2 cells exposed to increasing concentrations of each compound up to 400 μM; the results are shown in Figures 4 and 5, respectively, and are summarized in Table 1. Chlorin e_6 and its lysyl derivatives **3**, **7b**, and **8b** were found to be nontoxic in the dark up to the highest concentration (400 μM) investigated. All other amino acid derivatives showed very low dark cytotoxicities, with IC_{50} > 320 μM except for the 13^1-chlorin e_6 derivatives **9** and **13**, which showed IC_{50} of 285 and 268 μM, respectively. However, upon exposure to a low light dose (1 J/cm^2), all chlorin e_6 derivatives were found to be highly toxic to HEp2 cells (Figure 3). The most phototoxic were the 13^1-chlorin e_6 derivatives **9**, **11**, and **13**, with estimated IC_{50} values of 0.61, 0.82, and 1.34 μM, respectively. Among these, the most promising aspartyl derivatives for PDT applications are compounds **9** and **11** because they have the highest dark cytotoxicity/phototoxicity ratio > 466:1. The

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Table 2. Major (++) and Minor (+) Subcellular Sites of Localization for Chlorin e_6 and Its Derivatives in HEp2 Cells

compd	lysosomes	ER	Golgi	mitochondria
chlorin e_6 (**5**)	+	++	−	−
17^3-LysChlorin e_6TME (**3**)	+	++	+	−
15^2-AspChlorin e_6DME (**7a**)	++	++	−	+
15^2-LysChlorin e_6TME (**7b**)	++	++	+	−
15^2-AspPdChlorin e_6DME (**8a**)	++	+	++	−
15^2-LysPdChlorin e_6TME (**8b**)	+	++	−	−
13^1-AspChlorin e_6DME (**9**)	+	++	++	++
13^1-βAlaAspChlorin e_6DME (**11**)	++	++	++	−
13^1-EDLysChlorin e_6DME (**13**)	++	++	++	−

presence of the β-alanine spacer between the 13^1 carbonyl group and the aspartic acid residue seems to have only a small effect, slightly decreasing compound cytotoxicity. On the other hand, the 15^2-aspartylchlorin e_6 derivative **7a** was less phototoxic than its 13^1 regioisomer **9** by approximately 7-fold, and the introduction of palladium(II) further reduced its phototoxicity. The positively charged 17^3- and 15^2-lysylchlorin e_6 derivatives **3** and **7b** were the least phototoxic, and the introduction of palladium increased the phototoxicity of the 15^2 derivative by about 10-fold. These results show for the first time that the phototoxicity of amphiphilic conjugates of chlorin e_6 depends mainly on the site of conjugation, probably as a result of its effect on molecular conformation; the nature of the amino acid, the molecule overall charge, and the presence of palladium(II) also affect phototoxicity, but apparently to a smaller extent. Our results suggest that the most extended, nearly linear conformation of the 13^1 regioisomers probably facilitates binding to specific biological substrates, enhancing their toxic effect.

3.3. Intracellular Localization. The preferential sites of subcellular localization of this series of chlorin e_6 derivatives were evaluated by fluorescence microscopy upon exposure of HEp2 cells to 10 μM compound concentrations for 18 h. Figure S4 of the Supporting Information shows the fluorescence pattern observed for all compounds, and Table 2 summarizes their main sites of subcellular localization. Overlay experiments using the organelle specific fluorescence probes BODIPY Ceramide (Golgi), LysoSensor Green (lysosomes), MitoTracker Green (mitochondria), and ER Tracker Blue/White (ER) were conducted to evaluate the preferential sites of compound localization, as seen in Figures 6, 7, and 8 for the most phototoxic 13^1-regioisomers, respectively, and Figures S5−S10 of the Supporting Information. All chlorin e_6 derivatives **3**, **7a**, **7b**, **8a**, **8b**, **9**, **11**, and **13** were found to localize in the lysosomes and the ER (Table 2). This is not surprising because the structurally related 15^2-aspartylchlorin e_6 is a known lysosomal localizer, and HPPH (2-[1-hexyloxyethyl]-2-devinyl-pyropheophorbide *a*) localizes preferentially in the ER.[41] Photodamage to the ER and/or lysosomes has been shown to lead to activation of apoptotic pathways.[41−43] Furthermore, all the 13^1-chlorin e_6 regioisomers **9**, **11**, and **13** were also found in Golgi, and in addition the most phototoxic derivative **9** also localized in mitochondria; presumably, the photodamage effect to multiple organelles caused by the 13^1 derivatives can trigger various apoptotic pathways, leading to more effective cell destruction. The multiple sites of intracellular localization observed for the 13^1-chlorin e_6 derivatives might again be due to their linear conformations that facilitate their binding to various intracellular sites.

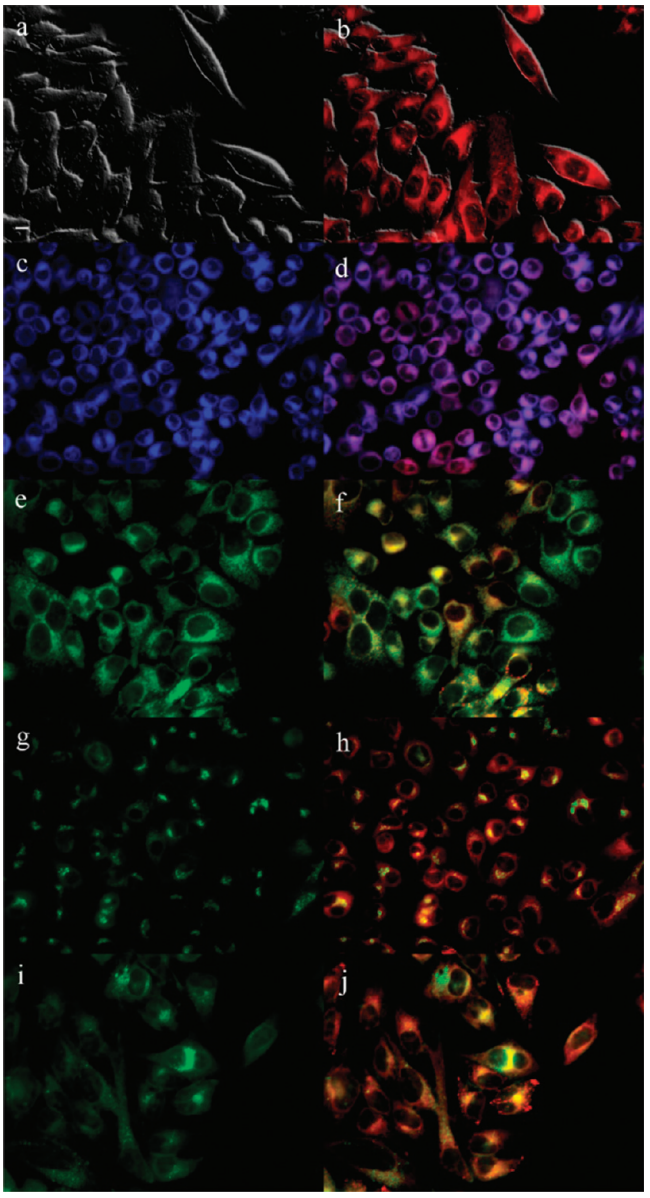

Figure 6. Subcellular localization of conjugate 13^1 AspCe$_6$DME (**9**) in HEp2 cells at 10 μM for 18 h (a) phase contrast, (b) overlay of conjugate **9** compound and phase contrast, (c) ER Tracker Blue/White fluorescence, (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d,f,h,j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μm.

CONCLUSIONS

A series of eight chlorin e_6 derivatives, conjugated with either aspartic acid or lysine residues at positions 17^3, 15^2, or 13^1 of the chlorin macrocycle, have been synthesized in good yields from pheophytin *a* (**1**). In comparison with chlorin e_6 (**5**), all amino acid derivatives readily accumulated within human HEp2 cells, and in particular the 15^2-lysyl derivatives were taken up by cells to a significantly higher extent than were all other regioisomers. On the other hand, the metal-free 15^2-lysylchlorin e_6 derivative (**7b**) showed the *least* phototoxicity, followed by the 17^3-lysyl regioisomer (**3**); insertion of palladium into 15^2-lysychlorin e_6 (**7b**) increased phototoxicity by approximately 9-fold. However, the most phototoxic compounds were found to be the 13^1-regioisomers, bearing

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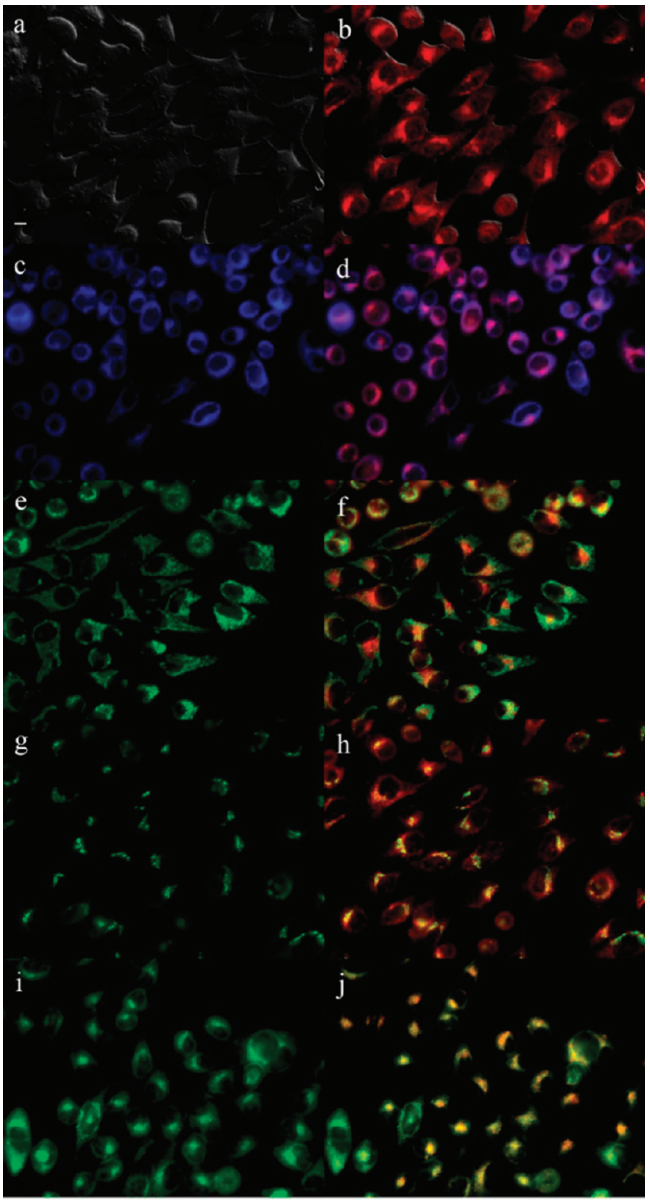

Figure 7. Subcellular localization of conjugate 13^1 β-Ala-AspCe_6DME (**11**) in HEp-2 cells at 10 μM for 18 h (a) phase contrast, (b) overlay of conjugate **11** and phase contrast, (c) ER Tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d,f,h,j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μm.

either an aspartic acid or a lysine residue directly conjugated to position-13 of the chlorin macrocycle or connected via a β-alanine or ethylene diamine spacer. The most phototoxic compound of this series, and the most promising for PDT applications, is 13^1-aspartylchlorin e_6 (**9**). Molecular modeling calculations show that the 13^1-regioisomers assume extended, nearly linear conformations that might facilitate their binding to multiple intracellular components and subsequent photodamage to multiple cellular sites. Therefore, the site of amino acid conjugation at the chlorin e_6 macrocycle is a major determinant of compound phototoxicity, and we hypothesize that the 13^1-regioisomer of mono-L-aspartylchlorin e_6 (**9**) might be a more efficient photosensitizer for PDT than is the commercial regioisomer (15^2-mono-L-aspartylchlorin e_6).

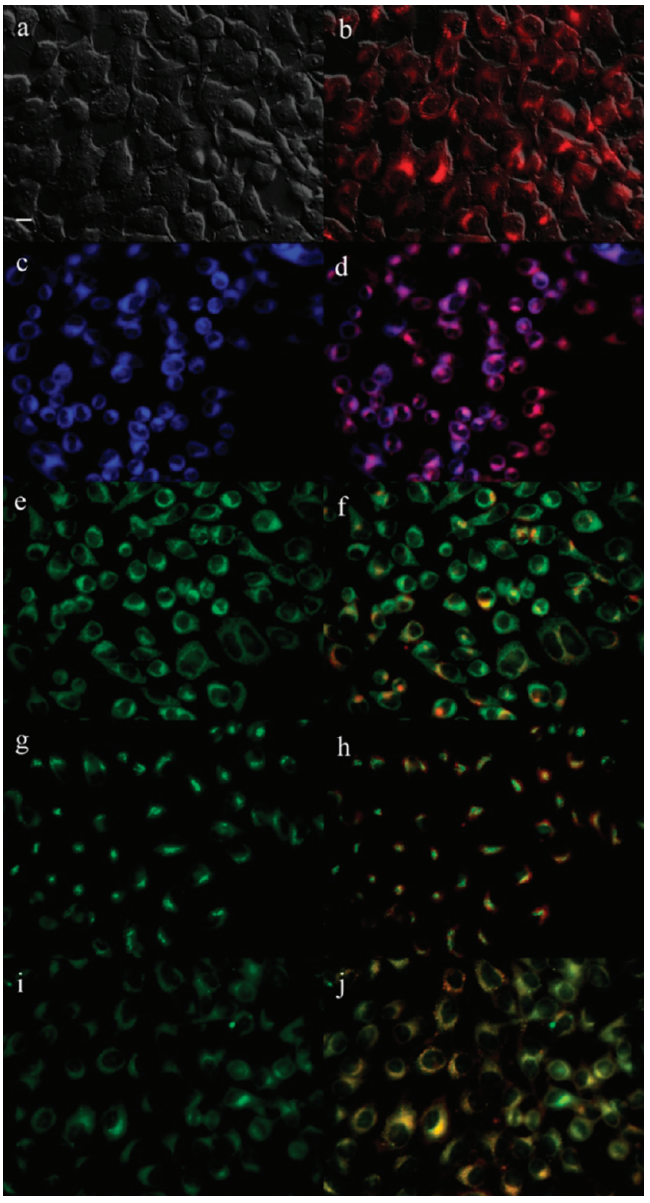

Figure 8. Subcellular localization of conjugate 13^1 ED-LysCe_6DME (**13**) in HEp-2 cells at 10 μM for 18 h (a) phase contrast, (b) overlay of conjugate **11** and phase contrast, (c) ER Tracker Blue/White fluorescence (e) MitoTracker Green fluorescence, (g) BoDIPY Ceramide, (i) LysoSensor Green fluorescence, and (d,f,h,j) overlays of organelle tracers with compound fluorescence. Scale bar: 10 μm.

EXPERIMENTAL SECTION

1. Chemistry. All air and moisture sensitive reactions were performed in dried and distilled solvents under an argon atmosphere. All solvents and reagents were purchased from commercial sources, unless otherwise stated. Silica gel 60 (230 × 400 mesh, Sorbent Technologies) was used for column chromatography. Analytical thin-layer chromatography (TLC) was carried out using polyester backed TLC plates 254 (precoated, 200 μm) from Sorbent Technologies. NMR spectra were recorded on an AV-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). The chemical shifts are reported in δ ppm using the following partially deuterated solvents as internal references: CD_2Cl_2 5.32 ppm - (^{1}H), 54 ppm (^{13}C); DMSO-*d* 2.49 ppm (^{1}H), 39.5 ppm (^{13}C); CH_3OH-*d* 4.78 ppm (^{1}H), 49.0 ppm (^{13}C); $CDCl_3$ 7.26 ppm (^{1}H),

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77.16 ppm (^{13}C); $(CH_3)_2CO$ 2.50 ppm (^{1}H), 29.84 ppm (^{13}C). Electronic absorption spectra were measured on an Agilent 8453 UV/vis spectrophotometer. Mass spectra were obtained on a Bruker Omniflex MALDI time-of-flight mass spectrometer. *Spirulina pacifica* alga was purchased as a spray-dried powder from Cyanotech, Hawaii. Pheophytin *a* (**1**) was extracted from *Spirulina pacifica* alga as previously published, and its spectroscopic characterization agreed with the published data.[29] All compounds synthesized were purified and isolated in ≥95% purity, as evidenced by analytical TLC in at least two solvent systems and confirmed by the absence of extraneous tetrapyrrole resonances in ^{1}H and ^{13}C NMR spectra. Energy minimization of all compounds was performed in the framework of Hartree−Fock. The restricted Hartree−Fock functional was used at the 6-31G level. All structures were optimized without any symmetry constrains. The solvent effects were accounted for using the Polarizable Continuum Model (PCM). All calculations were performed using the Gaussian 09 program package.

*17^3 Lysyl(Boc)OMe-pheophorbide a (**2**).* Pheophytin *a* (**1**) (250 mg, 0.29 mmol) was selectively hydrolyzed to the 17^3 carboxylic acid without affecting the 13^1 carbomethoxy group, as previously reported.[31] After evaporation of solvent, 155 mg (0.26 mmol), 90% of pheophorbide *a* ($C_{35}H_{36}N_4O_5$) was obtained. The spectroscopic data obtained for the compound agreed with published data. Pheophorbide *a* (100 mg; 0.169 mmol) was dissolved in 10 mL of CH_2Cl_2. Then DCC (42 mg) and DMAP (10 mg) were added and left for 15 min. H-Lys(Boc)OMe·HCl (60 mg) and DIEA (0.035 mL) were dissolved in 5 mL of CH_2Cl_2, added to the reaction mixture, and stirred for 4 h. [Note: this reaction is favored by dilute conditions; concentrated conditions favor the formation of the anhydride (bispheophorbide) and will slow the reaction]. The reaction mixture was washed with 10% citric acid, water, and brine, dried over anhydrous Na_2SO_4, and purified on a silica gel column (10% acetone in CH_2Cl_2). After the major brown band was eluted from the column, the solvent was evaporated and the solid was dissolved in ethyl acetate and filtered (DCC precipitated in ethyl acetate.) Evaporation of the filtered ethyl acetate gave 74 mg, 0.089 mmol, 52% yield of lysyl-(Boc)OMe-pheophorbide *a* ($C_{47}H_{58}N_6O_8$). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.12 (s, 1H), 8.71 (s, 1H), 8.70 (s, 1H), 7.64 (dd, J = 17.8, 11.63 Hz, 1H), 7.20 (d, J = 7.2 Hz, 1H), 6.33 (s, 1H), 6.02 (d, J = 17.85 Hz, 1H), 5.93 (d, J = 11.61 Hz, 1H), 5.77 (s, 1H), 4.65 (m, 1H), 4.41 (m, 1H), 4.21 (m, 1H), 3.91 (s, 3H), 3.59 (s, 3H), 3.47 (s, 3H), 3.19 (s, 3H) 3.09 (q, J = 7.5 Hz, 2H), 2.92 (3H, s), 2.83 (m, 2H), 2.66 (m, 1H), 2.48 (m, 1H), 2.25 (m, 1H), 1.85−1.62 (br, 4H), 1.86 (d, J = 7.24, 3H), 1.37 (t, J = 7.58, 3H), 1.43 (m, 2H), 1.26 (s, 9H), −1.95 (s, 1H), −2.23 (s, 1H).

*17^3 Lysyl-chlorin e$_6$ TME (**3**).* Compound **2** (74 mg, 0.089 mmol) was dissolved in dry 2:1 THF/MeOH (10 mL) and stirred under argon for 10 min. Sodium methoxide (0.17 mL of a 0.5 M solution) was added, and the reaction was allowed to stir at 0 °C for 1 h. The reaction was followed using spectrophotometry. The solution turned from brown to green as the isocyclic ring opens. The reaction mixture was then poured into water. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with water and 5% citric acid, dried over anhydrous Na_2SO_4, and then evaporated. The residue was dissolved in 2% MeOH/CH_2Cl_2 and purified on a silica gel plug with the same mobile phase. The solvent was evaporated, and 68 mg (0.078 mmol), 89% yield, of 17^3-lysyl(Boc)chlorin e$_6$ TME ($C_{48}H_{62}N_6O_9$) was obtained. ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.80 (s, 1H), 9.65 (s, 1H), 9.03 (s, 1H), 8.19 (dd, J = 17.87, 11.58 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 6.40 (d, J = 17.87, 1.33 Hz, 1H), 6.13 (d, J = 11.61, 1.36 Hz, 1H), 5.89 (s, 1H), 5.38 (m, 2H), 4.63 (q, J = 7.22 Hz, 1H), 4.55 (dd, J = 10.34, 1.95 Hz, 1H), 4.44 (m, 1H), 4.25 (s, 3H), 3.78 (q, J =7.67, 2H) 3.75 (s, 3H), 3.69 (s, 3H), 3.56 (s, 3H) 3.49 (s, 3H), 3.27 (3H, s), 3.00 (m, 2H), 2.66 (m, 1H), 2.33 (m, 1H), 2.20 (m, 1H), 1.85−1.62 (br, 4H), 1.78 (d, J = 7.24, 3H), 1.69 (t, J = 7.58, 3H), 1.43 (m, 2H), 1.32 (s, 9H), −1.32 (s, 1H), −1.52 (s, 1H). Lysyl(Boc)chlorin e$_6$ TME (68 mg, 0.078 mmol) was dissolved in 2 mL of dry CH_2Cl_2 in an ice bath under argon. Thioanisole (0.057 mmol) and 1 mL of TFA were added, and the reaction mixture was allowed to stir overnight. The reaction mixture was evaporated several times with diethyl ether to remove TFA. The resulting precipitate was washed several times with diethyl ether to remove thioanisole. The precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove last traces of TFA. The organic layer was dried over anhydrous Na_2SO_4. Solvent was evaporated, and 43 mg (0.056 mmol), 72% yield, of 17^3-lysylchlorin e$_6$ TME ($C_{43}H_{54}N_6O_7$) was obtained. UV−vis (acetone): λ_{max} (ε/M^{-1}cm^{-1}) 664 (65000), 608 (4871), 528 (4461), 500 (16100), 400 (213000). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.79 (s, 1H), 9.63 (s, 1H), 9.03 (s, 1H), 8.17 (dd, J = 17.87, 11.58 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 6.38 (d, J = 17.87, 1.33 Hz, 1H), 6.12 (d, J = 11.61, 1.36 Hz, 1H), 5.37 (m, 2H), 4.63 (q, J = 7.22 Hz, 1H), 4.55 (dd, J = 10.34, 1.95 Hz, 1H), 4.44 (m, 1H), 4.25 (s, 3H), 3.76 (m, 2H) 3.75 (s, 3H), 3.69 (s, 3H), 3.55 (s, 3H) 3.48 (s, 3H), 3.25 (3H, s), 3.09 (m, 2H), 2.67 (m, 1H), 2.30 (m, 1H), 2.20 (m, 1H), 1.85−1.62 (br, 3H), 1.78 (d, J = 7.24, 3H), 1.68 (t, J = 7.58, 3H), 1.51 (m, 2H), 1.39 (m, 2H), −1.33 (s, 1H), −1.53 (s, 1H). HRMS (MALDI-TOF) m/z 767.442 $[M + H]^+$, calcd for $C_{43}H_{55}N_6O_7$ 767.413.

*Chlorin e$_6$ (**5**).* Chlorin e$_6$ TME was prepared as previously published, and its spectroscopic characterization agreed fully with the published data.[22] Chlorin e$_6$ TME (50 mg, 0.078 mmol) was dissolved in anhydrous ethyl acetate (10 mL) under argon. Lithium iodide (124 mg, 0.94 mmol) was added. The reaction mixture was refluxed for 48 h under argon. The reaction mixture was diluted with water and adjusted to pH 3 with aqueous citric acid and then washed with CH_2Cl_2. The solution was evaporated, redissolved in acetone, and evaporated several times. The solid was washed with water and then dried under vacuum. The residue was dissolved in methanol and purified on a Sephadex LH-20 column to yield 10 mg, 0.017 mmol, 21% yield of chlorin e$_6$ ($C_{34}H_{36}N_4O_6$). UV−vis (MeOH): λ_{max} (ε/M^{-1}cm^{-1}) 666 (45271), 610 (8706), 558 (7835), 530 (9721), 502 (15525), 402 (145100). MS MALDI: m/z 597 $(M + H)^+$. ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.73 (s, 1H), 9.51 (s, 1H), 9.04(s, 1H), 8.05 (dd, J = 17.86, 11.61 Hz, 1H), 6.28 (dd, J = 17.85, 1.26 Hz, 1H), 6.02 (dd, J = 11.57, 1.37 Hz, 1H), 5.60 (d, J = 18.94 Hz, 1H), 5.40 (d, J = 18.94 Hz, 1H), 4.65 (m, 1H), 4.55 (m, 1H), 3.64 (m, 2H), 3.63 (s, 3H), 3.42 (s, 3H), 3.15 (s, 3H), 2.72 (m, 1H), 2.35 (m, 1H), 2.26 (m, 1H), 2.06 (m, 1H), 1.75 (d, J = 7.5 Hz, 3H), 1.64 (t, J = 7.5 Hz, 3H), −1.6 (s, 2H)

*Chlorin e$_6$ DME (**6**).* Chlorin e$_6$ DME was prepared as previously published, and its spectroscopic characterization agreed with the published data.[22]

*15^2 Aspartylchlorin e$_6$ DME (**7a**).* Chlorin e$_6$ (100 mg, 0.168 mmol) was dissolved in dry CH_2Cl_2. DCC (35 mg) and DMAP (9 mg) were added and allowed to stir until completely dissolved. After 3 h, H-Asp-$(OtBu)_2$·HCl (47.2 mg) and DIEA (0.029 mL) were mixed in CH_2Cl_2 and added to the reaction mixture. The reaction was allowed to stir overnight. The mixture was diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by washing with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was dissolved in CH_2Cl_2 and treated with ethereal diazomethane. The residue was dissolved in 2% MeOH/CH_2Cl_2 and purified via silica gel column chromatography using the same mobile phase to afford 65 mg, 0.076 mmol, 45% yield of 15^2-monoaspartylchlorin e$_6$ di(tert)butyl dimethyl ester ($C_{48}H_{61}N_5O_9$). MS (MALDI) m/z 853 $(M + H)^+$. ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.64 (s, 1H), 9.32 (s, 1H), 9.02 (s, 1H), 7.81 (dd, J = 11.58, 17.85 Hz, 1H), 7.00 (s, 1H), 6.09 (dd, J = 17.87, 1.26 Hz, 1H), 5.85 (dd, J = 11.60, 1.29 Hz, 1H), 5.38 (s, 2H), 4.6 (br, 3H), 4.32 (s, 3H), 3.62 (s, 3H), 3.54 (s, 3H), 3.52 (q, J = 7.3 Hz, 2H), 3.30 (s, 3H), 3.02 (s, 3H), 2.78 (m, 3H), 2.44 (m, 2H), 1.87 (m, 1H), 1.65 (d, J = 7.3 Hz, 3H), 1.58 (t, J = 7.7 Hz, 3H), 1.26 (s, 9H), 1.16 (s, 9H), −1.42 (s, 1H), −1.53 (s, 1H). The 15^2-Aspartylchlorin e$_6$ di(*tert*)butyl dimethyl ester (65 mg, 0.076 mmol) was dissolved in 2 mL of dry CH_2Cl_2 in an ice bath under argon. Thioanisole (0.006 mL) and

2 mL of TFA were added, and the reaction mixture was allowed to stir overnight. The reaction mixture was evaporated several times with diethyl ether to remove TFA. The resulting precipitate was washed several times with diethyl ether to remove thioanisole and then dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was washed with citric acid until all precipitate redissolved in the organic layer. The organic layer was dried over anhydrous Na_2SO_4. Solvent was evaporated, and 44 mg, 0.06 mmol, 79% yield of 15^2-aspartylchlorin e_6 DME was obtained ($C_{40}H_{45}N_5O_9$). UV−vis (acetone) λ_{max} (ε/$M^{-1}cm^{-1}$) 664 (48400), 609 (6000), 560 (2000), 529 (6280), 500 (14160), 402 (151800). MS (MALDI) m/z 841 $(M + H)^+$. ^1H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.76 (s, 1H), 9.58 (s, 1H), 9.04 (s, 1H), 8.10 (dd, J = 17.85, 11.59 Hz, 1H), 6.33 (dd, J = 17.87, 0.98 Hz, 1H), 6.07 (dd, J = 11.61, 1.09 Hz, 1H), 5.32 (m, 2H), 4.84 (m, 1H), 4.60 (m, 2H), 4.26 (s, 3H), 3.72 (q, J = 7.3 Hz, 2H), 3.57 (s, 3H), 3.54 (s, 3H), 3.45 (s, 3H), 3.21 (m, 3H), 2.95 (dd, J = 16.85, 5.53 Hz, 1H), 2.88 (dd, J = 16.85, 5.09 Hz, 1H) 2.68 (m, 1H) 2.32 (m, 2H), 1.78 (m, 1H), 1.74 (d, J = 7.3 Hz, 3H), 1.66 (t, J = 7.7 Hz, 3H), −1.39 (s, 1H), −1.58 (s, 1H). HRMS (MALDI-TOF) m/z 740.376 $[M + H]^+$, calcd for $C_{40}H_{46}N_5O_9$ 740.330

*15^2 Lysylchlorin e_6 TME (**7b**).* Chlorin e_6 (75 mg, 0.12 mmol) was dissolved in dry CH_2Cl_2 (10 mL). DCC (31.3 mg) and DMAP (11 mg) were added and allowed to stir until completely dissolved. After 3 h, H-lysyl(Boc)OMe·HCl (45 mg) and DIEA (0.022 mL) were mixed in CH_2Cl_2 and added to the reaction mixture. The reaction was allowed to stir overnight. It was then diluted with CH_2Cl_2 and washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue was dissolved in CH_2Cl_2 and treated with excess ethereal diazomethane. The residue was dissolved in 12% acetone/CH_2Cl_2 and purified via silica gel column chromatography with the same mobile phase to afford 84 mg, 0.097 mmol, 77% yield of 15^2-lysyl(Boc)chlorin e_6 TME ($C_{48}H_{62}N_6O_9$). MS (MALDI) m/z 868 $(M + H)^+$. ^1H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.77 (s, 1H), 9.55 (s, 1H), 9.04 (s, 1H), 8.08 (dd, J = 17.87, 11.58 Hz, 1H), 7.07 (m, 1H), 6.31 (dd, J = 17.87, 1.33 Hz, 1H), 6.06 (dd, J = 11.61, 1.36 Hz, 1H), 5.87 (s, 1H), 5.32 (s, 2H), 4.65 (q, J = 7.22 Hz, 1H), 4.60 (dd, J = 10.34, 1.95 Hz, 1H), 4.49 (m, 1H), 4.26 (s, 3H), 3.71 (q, J =7.67, 2H) 3.57 (s, 3H), 3.54 (s, 3H), 3.47 (s, 3H) 3.44 (s, 3H), 3.19 (3H, s), 2.97 (m, 2H), 2.71 (m, 1H), 2.37 (m, 2H), 1.80 (m, 3H), 1.77 (d, J = 7.24, 3H), 1.66 (t, J = 7.58, 3H), 1.39 (s, 9H), 1.27 (m, 2H), −1.35 (s, 1H), −1.56 (s, 1H). The 15^2-lysyl(Boc)chlorin e_6 TME (84 mg 0.097 mmol) was dissolved in 3 mL of dry CH_2Cl_2 in an ice bath under argon. Thioanisole (0.01 mL) and 1 mL of TFA were added, and the reaction mixture was allowed to stir overnight. The reaction mixture was evaporated several times with diethyl ether to remove residual TFA. The resulting precipitate was washed several times with diethyl ether to remove thioanisole. Then the precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was washed with citric acid until the precipitate was redissolved in the organic layer. The organic layer was dried over anhydrous Na_2SO_4 and then was evaporated to give 56 mg, 0.071 mmol, 73% yield of 15^2-lysylchlorin e_6 TME ($C_{43}H_{54}N_6O_7$). UV−vis (acetone) λ_{max} (ε/$M^{-1}cm^{-1}$) 664 (57800), 608 (4600), 529 (4900), 500 (14800), 400 (187200). ^1H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.68 (s, 1H), 9.42 (s, 1H), 9.02 (s, 1H), 7.95 (dd, J = 17.87, 11.58 Hz, 1H), 7.12 (d, J = 6.8 Hz, 1H), 6.20 (dd, J = 17.87, 1.33 Hz, 1H), 5.95 (dd, J = 11.61, 1.36 Hz, 1H), 5.35 (s, 2H), 4.65 (q, J = 7.22 Hz, 1H), 4.61 (dd, J = 10.34, 1.95 Hz, 1H), 4.53 (m, 1H), 4.27 (s, 3H), 3.59 (m, 2H) 3.55 (s, 6H), 3.48 (s, 3H) 3.37 (s, 3H), 3.09 (3H, s), 2.99 (m, 3H), 2.73 (m, 1H), 2.38 (m, 2H), 1.80 (m, 3H), 1.78 (d, J = 7.24, 3H), 1.61 (t, J = 7.58, 3H), 1.43 (m, 2H), −1.40 (s, 1H), −1.57 (s, 1H). HRMS (MALDI-TOF) m/z 767.399 $[M + H]^+$, calcd for $C_{43}H_{55}N_6O_7$ 767.413

*Palladium(II) 15^2-Aspartylchlorin e_6 DME (**8a**).* 15^2-Aspartylchlorin e_6 DME (44 mg, 0.06 mmol) was dissolved in 2 mL of dry THF. Palladium(II) acetate (14.2 mg, 0.063 mmol) was dissolved in THF and added to the reaction vessel and allowed to stir at 40 °C for 3 h. The reaction was followed by spectrophotometry. The solution turned from green to bluish−green as the complex formed. After evaporation of solvent, the residue was dissolved in methanol and purified via Sephadex LH-20 chromatography using the same mobile phase to afford 50 mg, 0.059 mmol, 98% yield of palladium 15^2-lysylchlorin e_6 TME ($C_{40}H_{43}N_5O_9Pd$). UV−vis (acetone): λ_{max} (ε/$M^{-1}cm^{-1}$) 619 (31240), 579 (6210), 489 (4035), 393 (44160). MS (MALDI) m/z 845 $(M + H)^+$. ^1H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.53 (s, 1H), 9.54 (s, 1H), 8.93 (s, 1H), 8.03 (dd, J = 17.82, 11.54 Hz, 1H), 6.17 (d, J = 17.79 Hz, 1H), 6.00 (dd, J = 11.61, 1H), 5.19 (d, J = 18.77 Hz, 1H), 5.04 (d, J = 18.59 Hz, 1H), 4.92 (m, 1H), 4.63 (m, 2H), 4.20 (s, 3H), 3.62 (s, 3H), 3.57 (m, 2H), 3.57 (s, 3H), 3.37 (s, 3H), 3.07 (m, 3H), 2.68 (m, 2H) 2.38 (m, 1H) 2.12 (m, 1H), 1.78 (m, 2H), 1.74 (d, J = 7.3 Hz, 3H), 1.50 (t, J = 7.7 Hz, 3H). HRMS (MALDI-TOF) m/z 843.109 $[M]^+$, calcd for $C_{40}H_{43}N_5O_9Pd$ 843.210.

*Palladium(II) 15^2-Lysylchlorin e_6 TME (**8b**).* 15^2-Lysylchlorin e_6 TME (56 mg) was dissolved in 5 mL of dry THF. Palladium(II) acetate (53 mg) was dissolved in THF and added to the reaction vessel and stirred at 60 °C for 3 h. The reaction was followed by spectrophotometry. The solution turned from green to bluish−green as the complex formed. After evaporation of solvent, the residue was dissolved in methanol and purified via Sephadex LH-20 chromatography using the same mobile phase to afford 61 mg, 0.07 mmol, 99% yield of palladium-(II) 15^2-lysylchlorin e_6 TME ($C_{43}H_{53}N_6O_7Pd$). UV−vis (acetone): λ_{max} (ε/$M^{-1}cm^{-1}$) 620 (97520), 581 (14500), 490 (7600), 394 (140900). ^1H NMR [$(CD_3)_2CO$, 400 MHz] all the peaks were broad due to partial paramagnetic nature of the compound. HRMS (MALDI-TOF) m/z 872.337 $[M + 2H]^+$, 767.450 $[M + H - Pd]^+$ calcd for $C_{43}H_{54}N_6O_7Pd$ 871.301, $C_{43}H_{55}N_6O_7$ 767.413.

*13^1 Aspartylchlorin e_6 DME (**9**).* Chlorin e_6 dimethyl ester (55 mg, 0.088 mmol) was dissolved in dry CH_2Cl_2. A mixture of HOBt (12 mg), TBTU (29 mg), and DIEA (0.017 mL) in DMF was added, and the mixture was allowed to stir for 30 min. H-Asp(OtBu)$_2$·HCl (60 mg) and DIEA (0.033 mL) were mixed in CH_2Cl_2 and added to this reaction mixture. The mixture was stirred overnight. It was diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was dissolved in 5% acetone/CH_2Cl_2 and purified via silica gel column chromatography using the same mobile phase to afford 50 mg, 0.058 mmol, 66% yield of 13^1-aspartylchlorin e_6 di(*tert*)butyl dimethyl ester ($C_{48}H_{61}N_5O_9$). MS (MALDI) m/z 852 $(M + H)^+$. ^1H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.79 (s, 1H), 9.70 (s, 1H), 9.10 (s, 1H), 8.39 (d, J = 7.93 Hz, 1H), 8.19 (dd, J = 11.58, 17.85 Hz, 1H), 6.37 (dd, J = 17.85, 1.23 Hz, 1H), 6.10 (dd, J = 11.87, 1.26 Hz, 1H), 5.71 (d, J = 18.95 Hz, 1H), 5.30 (m, 2H), 4.67 (q, J = 7.22 Hz, 1H), 4.49 (dd, J = 10.34, 1.95 Hz, 1H), 3.76 (q, J = 7.3 Hz, 2H), 3.74 (s, 3H), 3.64 (s, 3H), 3.61 (s, 3H), 3.50 (s, 3H), 3.27 (s, 3H), 3.16 (dd, J = 5.8, 0.78 Hz, 2H), 2.73 (m, 1H), 2.32 (m, 2H), 1.79 (m, 1H), 1.69 (m, 6H), 1.64 (s, 9H), 1.53 (s, 9H), −1.57 (s, 1H), −1.85 (s, 1H). The 13^1-Aspartylchlorin e_6 di(*tert*)butyl dimethyl ester (50 mg, 0.058 mmol) was dissolved in 2 mL of dry CH_2Cl_2 in a ice bath under argon. Thioanisole (0.005 mL) and 2 mL of TFA were added, and the reaction mixture was stirred overnight. The reaction mixture was evaporated several times with diethyl ether to remove residual TFA. The resulting precipitate was washed several times with diethyl ether to remove thioanisole. Then the precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to give 38 mg, 0.051 mmol, 88% yield of 13^1-aspartylchlorin e_6 DME ($C_{40}H_{45}N_5O_9$). UV−vis (acetone): λ_{max} (ε/$M^{-1}cm^{-1}$) 663 (126800), 607 (9000),

528 (7450), 500 (31540), 399 (385800). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.79 (s, 1H), 9.70 (s, 1H), 9.12 (s, 1H), 8.50 (d, J = 7.93 Hz, 1H), 8.19 (dd, J = 11.58, 17.85 Hz, 1H), 6.37 (d, J = 18.85 Hz, 1H), 6.10 (d, J = 11.87 Hz, 1H), 5.71 (d, J = 18.95 Hz, 1H), 5.45 (dd, J = 13.76, 5.80 Hz, 1H), 5.30 (d, J = 18.93 Hz, 1H), 4.67 (q, J = 7.22 Hz, 1H), 4.50 (dd, J = 10.34, 1.95 Hz, 1H), 3.76 (q, J = 7.3 Hz, 2H), 3.71 (s, 3H), 3.63 (s, 3H), 3.60 (s, 3H), 3.51 (s, 3H), 3.31 (dd, J = 5.76, 3.47 Hz, 2H), 3.26 (s, 3H), 2.70 (m, 2H), 2.31 (m, 2H), 1.79 (m, 1H), 1.71 (d, J = 7.24, 3H), 1.67 (t, J = 7.58, 3H), −1.57 (br s, 1H), −1.87 (s, 1H). HRMS (MALDI-TOF) m/z 740.344 $[M + H]^+$, calcd for $C_{40}H_{46}N_5O_9$ 740.330

*13^1 β-Alanylchlorin e$_6$ DME (**10**).* Chlorin e$_6$ DME (55 mg, 0.088 mmol) was dissolved in dry CH_2Cl_2. A mixture of HOBt (12 mg), TBTU (29 mg), and DIEA (0.017 mL) in DMF was added, and the mixture was stirred for 30 min. β-Alanyl(OtBu)·HCl (18 mg) and DIEA (0.017 mL) were mixed in CH_2Cl_2 and added to the reaction mixture. The mixture was then allowed to stir for overnight before being diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue was dissolved in 5% acetone/CH_2Cl_2 and purified via silica gel column chromatography using the same mobile phase to afford 45 mg, 0.06 mmol, 68% yield of 13^1-β-alanylchlorin e$_6$ (*tert*)butyl dimethyl ester ($C_{43}H_{53}N_5O_7$). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.70 (s, 1H), 9.63 (s, 1H), 9.01 (s, 1H), 8.17 (t, J = 5.71 Hz, 1H), 8.11 (dd, J = 11.58, 17.85 Hz, 1H), 6.31 (d, J = 18.85 Hz, 1H), 6.04 (d, J = 11.87 Hz, 1H), 5.65 (d, J = 18.95 Hz, 1H), 5.38 (d, J = 19.06 Hz, 1H), 4.66 (q, J = 7.22 Hz, 1H), 4.52 (dd, J = 10.34, 1.95 Hz, 1H), 4.02 (m, 1H), 3.09 (m, 1H), 3.77 (s, 3H), 3.68 (q, J = 7.3 Hz, 2H), 3.61 (s, 3H), 3.51 (s, 3H), 3.46 (s, 3H), 3.21 (s, 3H), 2.71 (m, 2H), 2.31 (m, 2H), 1.79 (m, 1H), 1.72 (d, J = 7.24, 3H), 1.65 (t, J = 7.58, 3H), 1.54 (s, 9H), −1.62 (s, 1H), −1.91 (s, 1H). The 13^1-β-alanylchlorin e$_6$ (*tert*)butyl dimethyl ester (45 mg, 0.059 mmol) was dissolved in 1.5 mL of dry CH_2Cl_2 in a ice bath under argon. Thioanisole (0.005 mL) and 1.5 mL of TFA were added, and the reaction mixture was stirred overnight before being diluted with CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to give 39 mg, 0.056 mmol, 95% yield of 13^1-β-alanylchlorin e$_6$ DME ($C_{39}H_{45}N_5O_7$). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.74 (s, 1H), 9.72 (s, 1H), 9.10 (s, 1H), 8.22 (dd, J = 11.58, 17.85 Hz, 1H), 8.15 (t, J = 5.71 Hz, 1H), 6.39 (d, J = 18.85 Hz, 1H), 6.12 (d, J = 11.87 Hz, 1H), 5.65 (d, J = 18.95 Hz, 1H), 5.37 (d, J = 19.06 Hz, 1H), 4.66 (q, J = 7.22 Hz, 1H), 4.52 (dd, J = 10.34, 1.95 Hz, 1H), 4.07 (m, 1H), 3.95 (m, 1H), 3.77 (s, 3H), 3.68 (q, J = 7.3 Hz, 2H), 3.61 (s, 3H), 3.53 (s, 3H), 3.52 (s, 3H), 3.28 (s, 3H), 2.69 (m, 2H), 2.31 (m, 2H), 1.79 (m, 1H), 1.72 (d, J = 7.24, 3H), 1.65 (t, J = 7.58, 3H), −1.62 (s, 1H), −1.91 (s, 1H).

*13^1 β-Alanyl-aspartylchlorin e$_6$ DME (**11**).* Chlorin e$_6$ DME (39 mg, 0.056 mmol) was dissolved in dry CH_2Cl_2. A mixture of HOBt (8 mg), TBTU (18 mg), and DIEA (0.017 mL) in DMF was added and stirred for 30 min. H-Asp(OtBu)$_2$·HCl (40 mg) and DIEA (0.025 mL) were mixed in CH_2Cl_2 and added to the reaction mixture. The mixture was stirred overnight before being diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was dissolved in 10% acetone/CH_2Cl_2 and purified via silica gel column chromatography using the same mobile phase to afford 45 mg, 0.048 mmol, 87% yield of 13^1-β-alanyl-aspartylchlorin e$_6$ di(*tert*)butyl dimethyl ester. ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.74 (s, 1H), 9.70 (s, 1H), 9.10 (s, 1H), 8.19 (dd, J = 11.58, 17.85 Hz, 1H), 8.11 (t, J = 5.66 Hz, 1H), 7.64 (d, J = 8.11 Hz, 1H), 6.37 (d, J = 19.18 Hz, 1H), 6.10 (d, J = 12.94 Hz, 1H), 5.66 (d, J = 19.03 Hz, 1H), 5.38 (d, J = 19.09 Hz, 1H), 4.77 (dt, J = 8.14, 5.74 Hz, 1H), 4.66 (q, J = 7.22 Hz, 1H), 4.52 (dd, J = 10.34, 1.95 Hz, 1H), 4.07 (m, 1H), 3.95 (m, 1H), 3.75 (s, 3H), 3.76 (q, J = 7.3 Hz, 2H), 3.60 (s, 3H), 3.53 (s, 3H), 3.50 (s, 3H), 3.27 (s, 3H), 2.80 (d, J = 2.57 Hz, 1H), 2.79 (d, J = 3.06 Hz, 1H), 2.31 (m, 2H), 1.79 (m, 1H), 1.71 (d, J = 7.24, 3H), 1.68 (t, J = 7.58, 3H), 1.43 (s, 9H), 1.42 (s, 9H), −1.61 (s, 1H), −1.91 (s, 1H). The 13^1-β-alanyl-aspartylchlorin e$_6$ di(*tert*)butyl dimethyl ester (45 mg, 0.048 mmol) was dissolved in 2 mL of dry CH_2Cl_2 in a ice bath under argon. Thioanisole (0.004 mL) and 2 mL of TFA were added, and the reaction mixture was stirred overnight before being evaporated several times with diethyl ether to remove residual TFA. The resulting precipitate was washed several times with diethyl ether to remove more residual thioanisole. The precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to give 38 mg, 0.046 mmol, 97% yield of 13^1-aspartylchlorin e$_6$ DME ($C_{43}H_{50}N_6O_{10}$). UV−vis (acetone): λ_{max} (ε/M^{-1}cm^{-1}) 663 (77125), 607 (3621), 528 (1831), 500 (17200), 399 (237100). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.72 (s, 1H), 9.70 (s, 1H), 9.10 (s, 1H), 8.20 (dd, J = 11.58, 17.85 Hz, 1H), 8.11 (br t, J = 5.66 Hz, 1H), 7.64 (br d, J = 8.11 Hz, 1H), 6.32 (d, J = 19.18 Hz, 1H), 6.10 (d, J = 12.94 Hz, 1H), 5.63 (d, J = 19.03 Hz, 1H), 5.37 (d, J = 19.09 Hz, 1H), 4.92 (dt, J = 8.14, 5.74 Hz, 1H), 4.66 (q, J = 7.22 Hz, 1H), 4.51 (dd, J = 10.34, 1.95 Hz, 1H), 4.07 (m, 1H), 3.95 (m, 1H), 3.74 (s, 3H), 3.75 (q, J = 7.3 Hz, 2H), 3.60 (s, 3H), 3.51 (s, 3H), 3.50 (s, 3H), 3.25 (s, 3H), 2.96 (d, J = 5.27 Hz, 1H), 2.69 (d, J = 3.06 Hz, 1H), 2.31 (m, 2H), 1.79 (m, 1H), 1.71 (d, J = 7.24, 3H), 1.66 (t, J = 7.58, 3H), −1.61 (s, 1H), −1.91 (s, 1H). HRMS (MALDI-TOF) m/z 811.381 $[M + H]^+$, calcd for $C_{43}H_{51}N_6O_{10}$ 811.367.

*13^1 Ethylenediaminylchlorin e$_6$ DME (**12**).* Methyl pheophorbide *a*, (100 mg, 0.164 mmol) was dissolved in dry $CHCl_3$ and stirred under argon for 10 min. Then 0.2 mL of ethylenediamine was added to the solution and the mixture stirred for 24 h. The reaction was monitored by spectrophotometry. The reaction mixture was evaporated, and the residue was dissolved in 2.5% MeOH/CH_2Cl_2 and then chromatographed on a short silica gel column using the same mobile phase to remove byproduct, and then the product was eluted using 50% MeOH/CH_2Cl_2 to afford 100 mg, 0.150 mmol, 91% yield of 13^1-ethylenediaminylchlorin e$_6$ DME ($C_{38}H_{46}N_6O_5$). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.69 (s, 1H), 9.63 (s, 1H), 9.09 (s, 1H), 8.09 (dd, J = 17.78, 11.58 Hz, 1H), 8.07 (br s, 1H), 6.29 (d, J = 18.85 Hz, 1H), 6.02 (d, J = 11.87 Hz, 1H), 5.67 (d, J = 19.08 Hz, 1H), 5.39 (d, J = 19.11 Hz, 1H), 4.66 (q, J = 7.22 Hz, 1H), 4.51 (dd, J = 10.34, 1.95 Hz, 1H), 4.01 (m, 1H), 3.87 (m, 1H), 3.75 (s, 3H), 3.66 (q, J = 7.3 Hz, 2H), 3.61 (s, 3H), 3.50 (s, 3H), 3.45 (s, 3H), 3.20 (s, 3H), 2.71 (m, 1H), 2.31 (m, 2H), 1.95 (br m, 1H), 1.80 (m, 1H), 1.72 (d, J = 7.24, 3H), 1.64 (t, J = 7.58, 3H), −1.64 (s, 1H), −1.93 (s, 1H). ^{13}C NMR [$(CD_3)_2CO$, 100 MHz] δ 173.1, 173.0, 169.2, 168.3, 167.7, 153.6, 149.1, 144.4, 138.1, 136.0, 135.3, 134.7, 133.9, 130.1, 129.8, 129.3, 120.9, 103.2, 100.7, 98.4, 93.9, 53.1, 51.4, 50.8, 50.2, 48.8, 41.2, 37.2, 30.7, 29.6, 22.6, 19.0, 17.2, 11.3, 11.0, 10.3. HRMS (MALDI-TOF) m/z 667.395 $[M + H]^+$, calcd for $C_{38}H_{47}N_6O_5$ 667.361.

*13^1 Ethylenediaminyl-lysylchlorin e$_6$ DME (**13**).* (Boc)Lysine(Boc)OH (55 mg, 0.082 mmol) was dissolved in dry CH_2Cl_2 (10 mL) A mixture of HOBt (15 mg), TBTU (33 mg), and DIEA (0.025 mL) in DMF was added and then stirred for 1 h. 13^1-Ethylenediaminylchlorin e$_6$ DME (50 mg, 0.075 mmol) was added to the reaction mixture, which was stirred overnight. The mixture was diluted with CH_2Cl_2 and then washed with 5% aqueous citric acid, followed by a wash with brine and water. It was dried over anhydrous Na_2SO_4 and then evaporated. The residue was dissolved in 2.5% MeOH/CH_2Cl_2 and purified via silica gel column chromatography using the same mobile phase to afford 65 mg, 0.065 mmol, 78% yield of 13^1-ethylenediaminyl(Boc)lysyl(Boc)chlorin e$_6$ DME ($C_{54}H_{74}N_8O_{10}$). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.54 (s, 1H), 9.53 (s, 1H), 9.07 (s, 1H), 8.14 (br s, 1H) 7.94 (dd, J = 17.78, 11.58 Hz, 1H), 7.78 (br s, 1H), 6.18 (d, J = 18.85 Hz, 1H), 6.16 (br s, 1H), 5.93 (d, J = 11.87 Hz, 1H), 5.89 (br s, 1H), 5.63 (d, J = 19.08 Hz, 1H), 5.39 (d, J = 19.11 Hz, 1H), 4.67 (q, J = 7.22 Hz, 1H), 4.52 (dd, J = 10.34, 1.95 Hz, 1H), 4.17 (m, 1H), 3.85 (m, 1H),

3.75 (s, 3H), 3.66 (m, 3H), 3.61 (s, 3H), 3.52 (br q, J = 7.14 Hz, 2H), 3.41 (s, 3H), 3.39 (s, 3H), 3.11 (s, 3H), 3.00 (m, 2H), 2.71 (m, 1H), 2.32 (m, 2H), 1.95 (br m, 1H), 1.84 (m, 2H), 1.72 (d, J = 7.24, 3H), 1.67 (m, 1H), 1.59 (t, J = 7.58, 3H), 1.41 (m, 3H), 1.37 (s, 9H), 1.34 (s, 9H), −1.66 (s, 1H), −1.94 (s, 1H). The 13^1-ethylenediaminyl(Boc)lysyl(Boc)-chlorin e_6 DME (65 mg, 0.065 mmol) was dissolved in 3 mL of dry CH_2Cl_2 in a ice bath under argon. Thioanisole (0.003 mL) and 2 mL of TFA were added, and the reaction mixture was stirred overnight. The reaction mixture was evaporated several times with diethyl ether to remove TFA. The resulting precipitate was washed several times with diethyl ether to remove thioanisole. Then the precipitate was dissolved in CH_2Cl_2 and washed three times with H_2O and once with 10% $NaHCO_3$ to remove TFA. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated to give 42 mg, 0.052 mmol, 81% yield of 13^1-ethylenediaminyl-lysylchlorin e_6 DME ($C_{44}H_{58}N_8O_6$). UV−vis (acetone): λ_{max} (ε/$M^{-1}cm^{-1}$) 663 (76420), 607 (1810), 527 (657), 500 (14,560), 399 (242,100). ^{1}H NMR [$(CD_3)_2CO$, 400 MHz]: δ 9.76 (s, 1H), 9.69 (s, 1H), 9.10 (s, 1H), 8.29 (br s, 1H) 8.17 (dd, J = 17.78, 11.58 Hz, 1H), 7.86 (br s, 1H), 6.35 (d, J = 18.85 Hz, 1H), 6.07 (d, J = 11.87 Hz, 1H), 5.64 (d, J = 19.08 Hz, 1H), 5.40 (d, J = 19.11 Hz, 1H), 4.66 (q, J = 7.22 Hz, 1H), 4.52 (dd, J = 10.34, 1.95 Hz, 1H), 3.92 (m, 2H), 3.75 (br m, 5H), 3.74 (s, 3H), 3.60 (s, 3H), 3.53 (s, 3H), 3.49 (s, 3H), 3.26 (s, 3H), 2.96 (br t, 2H), 2.70 (m, 1H), 2.29 (m, 2H), 1.95 (br m, 1H), 1.84 (m, 2H), 1.72 (d, J = 7.24, 3H), 1.68 (t, J = 7.58, 3H), 1.45 (m, 2H), 1.31 (m, 2H) −1.60 (s, 1H), −1.89 (s, 1H). HRMS (MALDI-TOF) m/z 795.492 $[M + H]^+$, calcd for $C_{44}H_{59}N_8O_6$ 795.456.

2. Cell Studies. Human carcinoma HEp2 cells were maintained in a 50:50 mixture of DMEM:AMEM (Invitrogen) supplemented with 5% FBS (Invitrogen), 1% Primocin antibiotic (Invitrogen) in a humidified, 5% CO_2 incubator at 37 °C. The cells were subcultured twice weekly to maintain subconfluent stocks. The fourth to fifteenth passage cells were used for all experiments.

2.1. Time-Dependent Cellular Uptake. The HEp2 cells were plated at 7500 cells per well in a Costar 96-well plate and allowed to grow for 48 h. Compound stock solutions were prepared at 32 mM in DMSO and Cremophor (10% of Cremophor in DMSO). Further dilution into the cells of the 96-well plate gave a final concentration of 10 μM with maximum DMSO concentration of 1% and Cremophor concentration of 0.1%. For the uptake test, the compounds were diluted to 20 μM (2× stock solution) and added to the 96-well to give a final concentration of 10 μM at 0, 1, 2, 4, 8, 12, and 24 h interval. The uptake was terminated by removing the loading medium and washing the wells with 200 μL of PBS buffer. Cells and compounds were then solubilized using 100 μL of 0.25% Triton X-100 in PBS buffer. The compound concentration was measured using intrinsic fluorescence as measured with a BMG FLUOstar plate reader equipped with a 355 nm excitation and a 650 nm emission filter. The cells were measured using a CyQuant Cell proliferation assay (Invitrogen) as per manufacturer's instructions, as previously reported.[20,21,24]

2.2. Dark Cytotoxicity. The HEp2 cells were plated as described above for the uptake experiment. The compounds were diluted into media to give 400 μM solution concentrations. Two-fold serial dilutions were then prepared from 400 to 50 μM, and the cells were incubated for 18 h. The loading medium was removed, and the cells were fed new medium, followed by 20 μL of CellTiter blue per 100 μL of medium and incubated for 4 h. Cell toxicity was measured using Promega's CellTiter Blue Viability Assay Kit as per manufacturer's instructions; untreated cells were considered 100% viable and cells treated with 0.2% saponin as 0% viable, as previously reported.[20,21,24]

2.3. Phototoxicity. The cells were prepared as described above with compound concentration range from 6.25 to 100 μM. After loading overnight, the medium was replaced with medium containing 50 mM HEPES pH 7.2. The cells were exposed to a 100 W halogen lamp filtered through a 610 nm long pass filter to provide approximately 1 J cm^{-2} light dose. The cells were kept cool by filtering the IR radiation through 10 mm of water and placing the culture in an ice−water bath. After exposure to light for 20 min, the plate was incubated in the 37 °C, 5% CO_2 incubator overnight. Cell viability was then measured as described above using CellTiter blue Viability Assay Kit, as previously reported.[20,21,24]

2.4. Microscopy. The cells were incubated in a glass bottom 6-well plate (MatTek) and allowed to grow for 48 h. The cells were then exposed to 10 μM of each compound for 18 h. Organelle tracers were obtained from Invitrogen and used at the following concentrations: LysoSensor Green 50 nM, MitoTracker Green 250 nM, ER Tracker Blue/white 100 nM, and BODIPY FL C5 ceramide 1 mM. After 30 min incubation in the 37 °C, 5% CO_2 incubator, both the media and the organelle tracers were removed and washed with PBS buffer for 3 times. Images were acquired using a Leica DMRXA microscope with 40× NA 0.8 dip objective lens and DAPI, GFP, and Texas Red filter cubes (Chroma Technologies).

■ ASSOCIATED CONTENT

Supporting Information. Normalized absorption spectra, energy minimized conformations in water, time-dependent uptake, and subcellular localization microscopy figures. Proton NMR spectra for compounds **2**−**13** and other synthetic intermediates, carbon-13 NMR shifts, carbon-13 NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS LIST

PS, photosensitizer; PDT, photodynamic therapy; DMF, dimethylformamide; DMSO, dimethylsulfoxide; THF, tetrahydrofuran; DME, dimethyl ester; TME, trimethyl ester; DBU, 1, 8-diazabicyclo[5.4.0]undec-7-ene; DCC, 1,3-dicyclohexylcarbodiimide; TFA, trifluoroactic acid; DIEA, *N*,*N*-diisopropylethylamine; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; DMAP, 4-dimethylaminopyridine; HOBt, 1-hydroxybenzotriazole; PBS, phosphate buffered saline; FBS, fetal bovine serum; MEM, modified eagle medium; ER, endoplasmic reticulum

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