Synthesis, Comparative Photosensitizing Efficacy, Human Serum Albumin (Site II) Binding Ability, and Intracellular Localization Characteristics of Novel Benzobacteriochlorins Derived from *vic*-Dihydroxybacteriochlorins

Guolin Li,[†] Andrew Graham,[‡] Yihui Chen,[†] Mahabeer P. Dobhal,[†,||] Janet Morgan,[‡] Gang Zheng,[†] Andrei Kozyrev,[†] Allan Oseroff,[‡] Thomas J. Dougherty,[†] and Ravindra K. Pandey[†,§,*]

Photodynamic Therapy Center, Department of Dermatology, Department of Nuclear Medicine and Radiology, Roswell Park Cancer Institute, Buffalo, New York 14263

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In a sequence of reactions, methyl mesopyropheophorbide *a*, mesochlorin e_6 trimethyl ester, mesochlorin p_6 trimethyl ester, mesopurpurin-18-*N*-hexylimide methyl ester, and mesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide methyl ester were synthesized from chlorophyll-a. These chlorins on reacting with osmium tetraoxide produced the corresponding *vic*-dihydroxybacteriochlorins. The 8-vinylchlorins obtained by refluxing the related *vic*-dihydroxybacteriochlorins in *o*-dichlorobenzene were individually treated with dimethylacetylenedicarboxylate (DMAD) under Diels−Alder reaction conditions. The intermediate adducts on 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) treatment rearranged to the corresponding stable benzobacteriochlorins, exhibiting the longest wavelength absorption in the range of 737 to 805 nm. In preliminary in vitro (RIF tumor cells) and in vivo screening (C3H/HeJ mice bearing RIF tumors), some of these compounds were found to be quite effective. Under similar treatment conditions (drug dose: 5.0 μmol/kg; light dose: 135 J/cm^2, tumors were exposed to light for 30 min at 24 h postinjection), the benzobacteriochlorins containing N-substituted-imide ring system produced enhanced photosensitizing efficacy with limited skin phototoxicity. These compounds were also found to bind to site II of human serum albumin (HSA). However, no correlation between the binding constant values and photosensitizing efficacy was observed. A competitive intracellular localization study of these novel structures with Rhodamine-123 (a mitochondrial probe) indicated their preferential localization in mitochondria, without producing any specific displacement of ^{3}H-PK11195 (PBR probe, ^{3}H-labeled 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide). These results suggest that the mitochondrial peripheral benzodiazepine receptor (PBR) is not the cellular binding site for this class of compounds.

Introduction

Among tetrapyrrolic systems, chlorins and bacteriochlorins have been proposed as potentially useful candidates for the use in photodynamic therapy (PDT) where strong absorption in the visible or near-IR region of the spectrum can be used to photoactivate dyes previously located in targeted (neoplastic) tissues.[1] Photoactivations of compounds at long-wavelength absorption (700−800 nm) may be able to treat larger tumors. Some naturally occurring bacteriochlorins have previously been reported as effective photosensitizers both in vitro and in vivo.[2] However, most of them are extremely sensitive to oxidation, which results in rapid transformation to the chlorin state (λ_{max} 640 nm).[2] Furthermore, if a laser is used to excite the bacteriochlorin in vivo, oxidation may result in the formation of a new chromophore absorbing outside the laser window, thus reducing the photodynamic efficacy. To render PDT more applicable to tumor therapy, there is need for long wavelength absorbing and stable photosensitizers that show the ability to localize at the tumor site in high concentrations and may be able to treat large tumors.

In recent years, several approaches have been quite successful for preparing stable bacteriochlorins exhibiting long wavelength absorption near-IR region. The first approach describes the in situ conversion of bacteriochlorophyll-a into bacteriopurpurin-18 methyl ester,[3] which under appropriate reaction conditions can be converted into a highly stable (both in vitro and in vivo) bacteriopurpurinimide system.[4] The second approach is to prepare a series of metallobacteriochlorophylls in which the central magnesium metal is replaced with other diamagnetic metals.[5] Some of the compounds in these two series are reported to be effective photosensitizers.[4,5] The third approach deals with the utility of Diels−Alder reaction for the preparation of stable bacteriochlorins. For example, it has been shown that pyrrole units containing vinyl groups at the diagonal positions of the porphyrin molecule on subjecting to double Diels−Alder reaction could be converted into novel bacteriochlorins.[6,7] These compounds exhibit long-wavelength absorption near 800 nm but produced limited PDT efficacy in mice implanted with RIF tumors. For developing a general synthesis of stable bacteriochlorins, Morgan et al.[8] converted the octaeth-

* To whom correspondence should be addressed. Phone: 716-845-3203. Fax: 716-845-8920. E-mail: ravindra.pandey@roswellpark.org.
[†] Photodynamic Therapy Center.
[‡] Department of Dermatology.
[§] Department of Nuclear Medicine and Radiology.
[||] On leave from the Department of Chemistry, University of Rajasthan, Jaipur 302004, India.

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ylporphyrins to the corresponding ketochlorins by following the pinacol−pinacolone approach, which in a sequence of reaction was converted into the corresponding vinylchlorin as a mixture of two positional isomers. These isomers were individually reacted with DMAD, and the corresponding bacteriochlorins were isolated in good yields with long wavelength absorption near 700 nm. The spectroscopic properties of these compounds resemble those of porphyrinones rather than bacteriochlorins. In preliminary in vivo screening these compounds produced limited in vivo efficacy. Recently Cavaleiro et al.[9] have shown that tetraphenylporphyrin (TPP) can be converted into a mixture of pyrrolidine-fused chlorin, isobacteriochlorin, and bacteriochlorin on refluxing with *N*-methylglycine and paraformaldehyde. This procedure, however, produced the desired bacteriochlorin (λ_{max} 732 nm) as a minor product. The same authors[10] also reported the synthesis of glycoconjugated isoxazolidine-fused bacteriochlorins by heating the mixture of porphyrin with an excess of sugar nitrone, and the resulting bacteriochlorin was isolated in low yield (about 9%). Bonnet et al.[11] extended diimide approach that had been used for the preparation of *m*-THPC (*m*-tetrahydroxyphenylporphyrin), for converting the meso-substituted porphyrins into the corresponding bacteriochlorins.

Chang et al.[12] have previously shown that octaethylchlorin on reacting with osmium tetraoxide produce the corresponding *vic*-dihydroxybacteriochlorin system in excellent yield. Roswell Park and University of California−Davis groups together extended this methodology to the pheophorbide *a* and chlorin e_6 series,[13] and the resulting bacteriochlorins exhibited strong absorption in the red region of the electronic spectra (730−750 nm). Unfortunately, these compounds did not produce any significant in vivo photosensitizing activity.[14] In our attempt to prepare 8-vinyl chlorins, we investigated the stability of the *vic*-dihydroxybacteriochlorins under various conditions and the formation of the corresponding desired product was found to depend on the reaction conditions used.[15−17] We extended the thermolysis approach using 1,2-dichlorobenzene as a solvent for the preparation of 8-vinylpurpurin-18 methyl ester which on reacting with dimethyl acetylenedicarboxylate (DMAD) under Diels−Alder reaction conditions produced benzobacteriopurpurin with long wavelength absorption at 795 nm.[18] Unfortunately, the six-membered fused anhydride ring system present in this molecule was found to be unstable in vivo and produced the corresponding chlorin p_6 analogue with a significant blue shift in its in vivo absorption.[19]

The present work describes the synthesis and biological evaluation of a series of benzobacteriochlorins derived from the related 8-vinylchlorins which are stable in vivo. Under Diels−Alder reaction conditions these chlorins were converted into the corresponding stable bacteriochlorins exhibiting long wavelength absorption in the range of 737−805 nm.

Results and Discussion

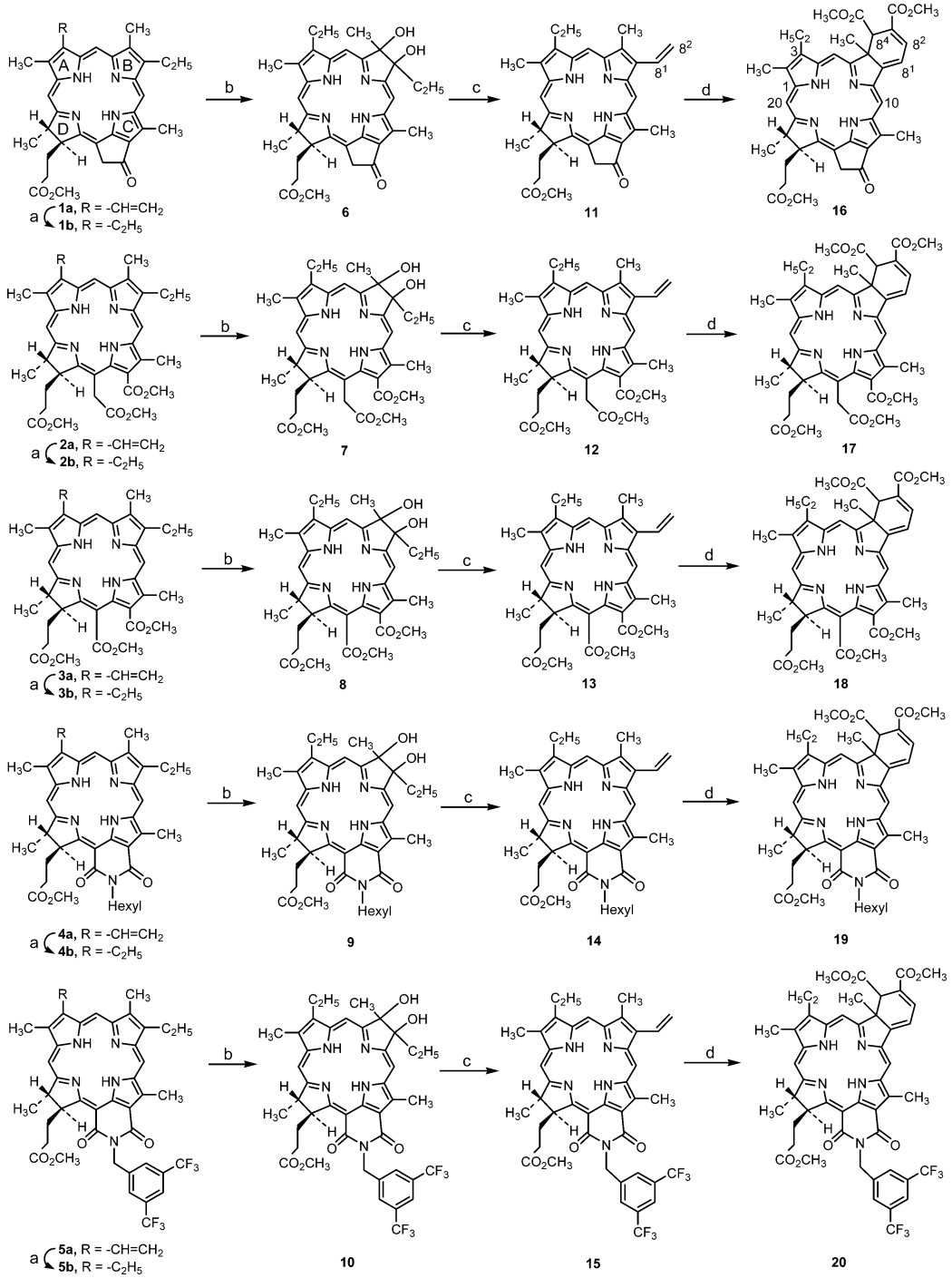
Chemistry. In this study, methyl mesopyropheophorbide *a* **1b**, mesochlorin e_6 trimethyl ester **2b**, mesochlorin p_6 trimethyl ester **3b**, methyl mesopurpurin-18-*N*-hexylimide **4b**, and methyl mesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide **5b** were used as substrates and were prepared by following the literature procedures.[20,21] These compounds were converted into the corresponding bacteriochlorins **16**−**20** by following the reaction sequences depicted in Scheme 1. For example, methyl mesopyropheophorbide *a* **1b** obtained from methylpyropheophorbide *a* **1a** was reacted with OsO_4, and the corresponding *vic*-dihydroxybacteriochlorin **6** was obtained in 65% yield as a mixture of two isomers (*cis*-hydroxy groups up or down relative to ring D). Bacteriochlorin **6** on refluxing in *o*-dichlorobenzene for 1.5 h produced 8-vinyl pyropheophorbide *a* **11** in 54% yield. The structure of this 8-vinyl product **11** was confirmed by 2D NMR studies (H−H COSY and ROESY). Reacting **11** with DMAD in refluxing toluene under inert N_2 atmosphere produced the intermediate Diels−Alder adduct. The intermediate adduct[22] was isolated but not characterized and immediately reacted with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at room temperature to give the desired bacteriochlorin **16** in 36% converted yield in two steps. Following a similar approach, mesochlorin e_6 trimethyl ester **2b**, mesochlorin p_6 trimethyl ester **3b**, mesopurpurin-18-*N*-hexylimide methyl ester **4b**, and mesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide methyl ester **5b** were converted in to the related 8-vinyl analogues **12**−**15**, respectively. Reaction of these vinyl derivatives with DMAD as a dienophile produced the corresponding benzobacteriochlorins **17**−**20** (Scheme 1). The intermediate Diels−Alder adduct **21** (Scheme 2) (before converting it into benzobacteriochlorin **17**) was characterized by ^{1}H NMR, H−H COSY NMR, and HRMS analyses. Other Diels−Alder adducts (intermediate products) after purification were not characterized and immediately converted into the corresponding benzobacteriochlorins **16** and **18**−**20**.

At the thermolysis step, the *vic*-dihydroxybacteriochlorins **6**−**10** in refluxing *o*-dichlorobenzene though mainly produced the desired 8-vinylchlorins **11**−**15**, it also generated other byproducts in minor quantities. These products were identified as the related 8-ketochlorins as well as the corresponding symmetrical and/or unsymmetrical dimers.[23] The nature of the carbon−carbon linkages joining these chromophores in a dimeric form was found to be dependent on the substituents present at the peripheral position of the macrocycle.[23]

Benzobacteriochlorins **16**−**20** reported here were obtained as a mixture of diastereomers due to the formation of two new chiral centers at positions C-7 and C-8[4] generated after Diels−Alder reaction/DBU rearrangement. The structures of these benzobacteriochlorins were confirmed by ^{1}H NMR and HRMS spectra. The ^{1}H NMR signal assignment for benzobacteriochlorin **16** was achieved by 2D NMR studies (H−H COSY and ROESY), and the resonances for the substituents in other benzobacteriochlorins were assigned by analyzing their H−H COSY NMR spectra and comparing their ^{1}H NMR data with that of bacteriochlorin **16**.

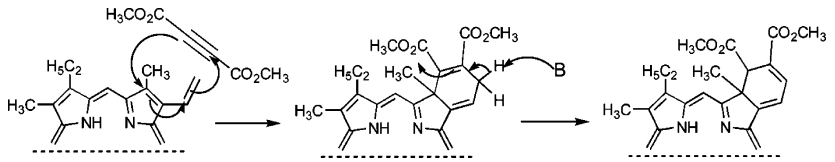
In the electronic absorption spectra, the long wavelength bands were observed in the range of 737−805 nm, and the shifts were significantly found to depend on the nature of the substituents present at the peripheral positions of the chromophore. The benzobacteriochlorins **19** and **20**, containing a fused-imide ring

Scheme 1[a]



[a] Reagents: a. (i) $Zn(OAc)_2$, (ii) H_2, Pd/C, (iii) TFA; b. OsO_4, H_2S; c. 1,2-dichlorobenzene; d. (i) DMAD, (ii) DBU.

Scheme 2



showed the maximum red shift and exhibited a strong absorption near 800 nm (Figure 1).

Biological Studies. Benzobacteriochlorins **16**−**20** were insoluble in water, and for biological studies, these compounds were formulated in 1% Tween 80/5% dextrose solution and filtered through a 0.22 μm syringe filter. The concentration of the photosensitizers in the filtrate was calculated on the basis of their extinction coefficient values, using the Beer−Lambert equation.[24] Compounds **16**−**20** were evaluated for both in vitro and

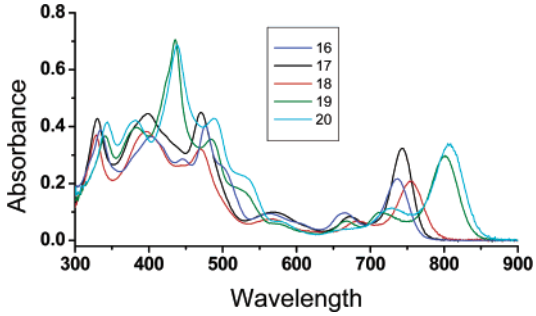


Figure 1. The electronic absorption spectra of benzobacteriochlorins **16**−**20** at a concentration of 10 μM in dichloromethane.

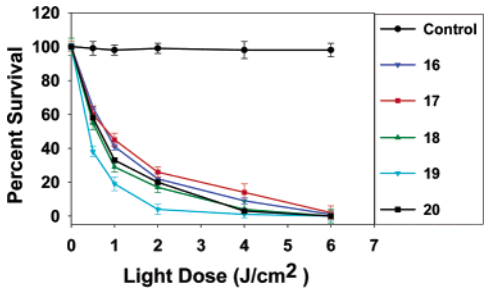


Figure 2. In vitro PDT efficacy of benzobacteriochlorin in RIF tumor cells at a dose of 1.0 μM at 48 h MTT. The cells were treated with light (737−805 nm, 0−6 J/cm^2, 18 mW/cm^2) after 4 h incubation (see the text). Control: Cells exposed to light without any photosensitizer. None of the photosensitizers produced any dark toxicity at 1.0 μM concentration (data not shown).

in vivo photosensitizing efficacy and a comparative study was also conducted to determine a possible correlation between the intracellular localization and human serum albumin Site II (benzodiazepine binding site) affinity to their photosensitizing efficacy.

In Vitro Photosensitizing Efficacy. Following the experimental details described previously,[4] benzobacteriochlorins **16**−**20** were tested for in vitro efficacy on radiation-induced fibrosarcoma (RIF) tumor cells at variable drug/light doses. A standard MTT assay was performed at a fixed drug concentration (1.0 μM), and the cells were exposed to variable light doses (1.0 to 6.0 J/cm^2) after 4 h incubation. [The optimal drug concentration for compound **19** was initially determined by evaluating the photosensitizing efficacy at variable concentration and the in vitro activity of other compounds was then compared under similar experimental conditions]. From the results summarized in Figure 2 it can be seen that among all the bacteriochlorins, compound **19** produced the best efficacy.

In Vivo Photosensitizing Activity. The in vivo efficacy of benzobacteriochlorins **16**−**20** was determined in C3H mice transplanted with RIF tumors (see Experimental Section). To determine the drug dose, the benzobacteriochlorin **19** that was found to be most effective in vitro was first evaluated at four different doses (1.0, 2.0, 3.5, and 5.0 μmol/kg) and the tumors (5 mice/group) were exposed to light (135 J/cm2) for 30 min at 24 h postinjection. The tumor growth (to reach > 400 mm^3) was monitored daily for 30 days. As can be seen from the results summarized in Figure 3, the best tumor response was observed at a dose of 5.0 μmol/kg (2/5 mice were tumor-free on day 30) and no apparent toxicity was

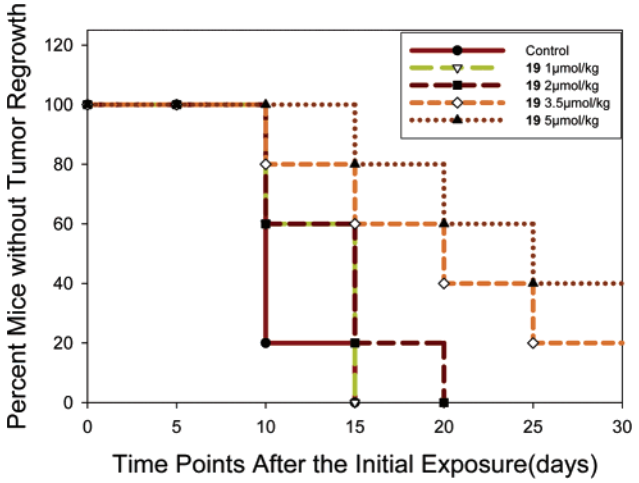


Figure 3. In vivo photosensitizing efficacy of benzobacteriochlorin **19** in mice (5 mice/group, bearing RIF tumors) at variable concentrations (1.0, 2.0, 3.5, and 5.0 μmol/kg). The tumors were exposed to a laser light (805 nm, 135 J/cm^2, 75 mWcm^2) at 24 h postinjection.

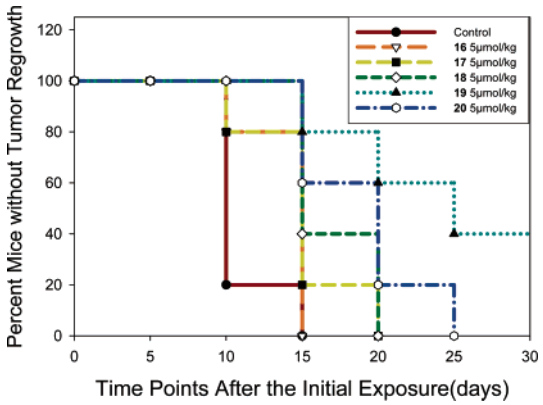


Figure 4. In vivo photosensitizing efficacy of benzobacteriochlorins **16**−**20** in C3H mice (5 mice/group) bearing RIF tumors at a dose of 5.0 μmol/kg. The mice were treated with laser light at their respective longest wavelength absorption (determined by in vivo reflectance spectroscopy) of the photosensitizer (737−805 nm, 135 J/cm^2, 75mWcm^2) at 24 h postinjection. Control: 12 mice were exposed to light without incubating the cells with photosensitizer.

observed. Therefore, for a comparative study, all bacteriochlorins **16**−**20** were then evaluated under similar treatment conditions and the results are depicted in Figure 4. As can be seen, compared to mice (5 mice/group) used for a control experiment, all photosensitizers showed significant delay in tumor regrowth. However, among all bacteriochlorins, compounds containing *N*-substituted-imide ring system **19** and **20** were most effective. For example, compound **19** produced 40% tumor response (2/5 mice were tumor free on day 30), and under similar treatment conditions bacteriochlorin **20** was slightly less effective, producing 20% tumor response (1/5 mice was tumor-free on day 25) but complete tumor regrowth was observed by day 30. Other compounds **16**−**18** showed some delay in tumor regrowth, but were certainly less effective than bacteriobenzochlorin **19**.

Skin Phototoxicity. The major problem associated with porphyrin-based compounds is long lasting skin phototoxicity.[25] Therefore, the phototoxicity of benzobacteriochlorinimide **19** at a dose of 5.0 μmol/kg was

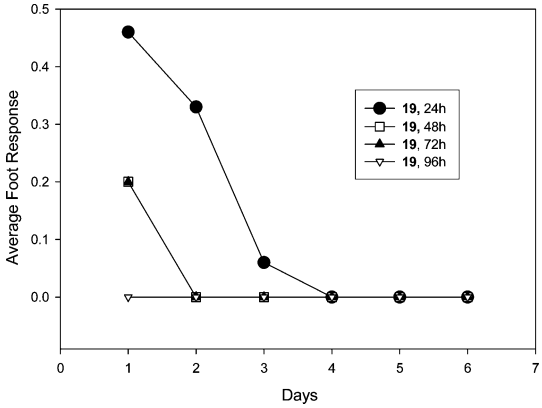


Figure 5. Skin phototoxicity vs days: Bacteriochlorin **19** at a dose of 5.0 μmol/kg was injected (iv) in12 mice (swiss mice, 4 mice/group). The hind foot of each mouse was exposed to light (similar to PDT conditions); the first group was exposed after 24 h postinjections and the other three groups at 48, 72, and 96 h postinjection, respectively. For details, see Results and Discussion).

investigated. For this study, bacteriochlorin **19** at a dose of 5.0 μmol/kg was injected (iv) to four groups of mice (Swiss mice, 3 mice/group). One of the hind feet of each mouse in the first group was exposed to light (at the therapeutic dose) at 24 h postinjection. The subsequent groups (3 mice/group) were exposed at 48, 72, and 96 h, respectively. In each group, the unexposed hind feet were used as control. Foot response was judged using a 0−3 scale: 0−0.1 = No apparent difference from normal, 0.3 = slight edema, 0.5 = moderate edema, 0.75 = large edema, 1.0 = large erythema with exudate, 1.2 = moderate edema with slightly crusty appearance, 1.5 = definite erythema, 1.65 = slightly damaged and or slight fusion of toes; 2.0 = most toes are fused but no change in general shape; 2.5 = foot shapeless with no toes, 3.0 = only stub of foot remaining. Response >2.0 indicates unacceptably severe normal tissue reaction.[25]

Compared to Photofrin at approximately an equieffective in vivo dose (8.3 μmol/kg) that shows slight damage and slight fusion of toes (score: 1.65−1.80), the benzobacteriochlorin **19** produced limited phototoxicity. Only a moderate edema (score: 0.5) was observed on day 1 at 24 h postinjection of the drug. At 48−72 h postinjection, no skin phototoxicity was observed, suggesting considerable tumor selectivity. The results are summarized in Figure 5.

Intracellular Localization. It has been shown that depending on the nature of the chromophore, effective photodynamic agents show very diverse patterns of localization, which also is based on lipophilicity, charge, and amphiphilicity. The predominant localization sites for most effective photosensitizers are reported to be mitochondria and/or the lysosomes.[26,27] The newly synthesized benzobacteriochlorins **16**−**20** with partition coefficient values in the range of 5.66−8.74 (**16**: 5.66, **17**: 6.25; **18**: 6.35; **19**, 7.13; and **20**, 8.74) were investigated for sites of localization by following the experimental procedure described previously.[4] All bacteriochlorins were found to localize to the same subcellular regions as Rhodamine-123, suggesting selectivity toward mitochondria (a representative example is shown in Figure 6).

Peripheral Benzodiazepine Receptor Binding Studies. In previous studies it has been implied by us and others that certain photosensitizers that show mitochondrial localization exhibit peripheral benzodiazepine receptor (PBR) binding which may be an important target for PDT.[28,29] Therefore, the ability of the newly synthesized benzochlorins to displace ^{3}H PK11195 (known PBR binding probe) from its specific cellular binding site [the intelligent quotient (IQ) site on PBR] was determined. Our preliminary experiments with increasing concentrations of mitochondrially localized benzobacteriochlorins **16**−**20** did not indicate any specific displacement of ^{3}H-PK11195. These results are in contrast to those obtained from the hexyl ether derivative of pyropheophorbide-a (HPPH)[28] and suggest that the PBR is not the target-site for the effective benzobacteriochlorin analogues (Figure 7).

Human Serum Albumin (Site II) Binding Studies. Human serum albumin (HSA) is the most abundant protein in human blood plasma. Many compounds, especially amphiphilic drugs and some endogenous substances, bind reversibly and with high affinity to HSA.[30] The formation of this complex decreases the concentration of unbound molecule in the plasma and thereby affects the ligand's distribution, pharmacokinetics, toxicity, and ultimately its rate of excretion. Previous studies on various porphyrin- and chlorin-based photosensitizers revealed there is a well-correlated relationship between photodynamic activity and HSA Site-II binding affinity to drugs, i.e. the photodynamically active compounds were generally found to bind to Site II of HSA.[31,32] Therefore, benzobacteriochlorins **16**−**20** were also subjected to HSA Site II binding ability following the literature procedure.[31,32] As can be seen from Table 1 and Figure 8 (only a representative example is shown), benzobacteriochlorins **16**−**20** competitively displaced DP (dansyl-L-proline), the Site II probe of HSA, with the binding constant values ranging from 1.06×10^7 to 1.75×10^7 M^{-1}. The binding constant values of benzobacteriochlorins **16**−**20** to HSA (Site II) were determined by fluorescence titration method. The theoretical basis and its application on our experiment had been described in detail

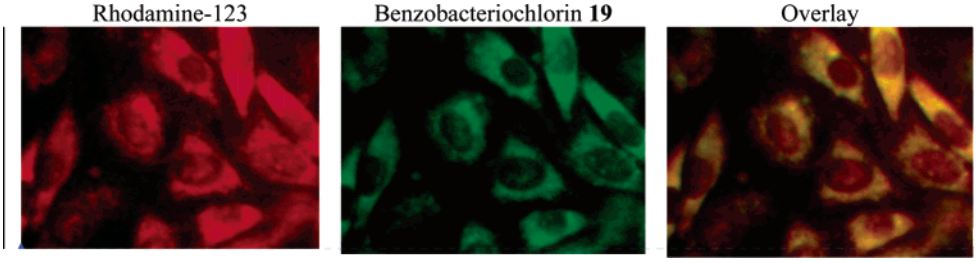


Figure 6. Comparative intracellular localization of benzobacteriochlorins **19** (1.0 μM) and Rhodamine123 (0.5 μM) in RIF tumor cells incubated for 24 and 0.5 h respectively. The images (false colors) were obtained by fluorescence light microscopy.

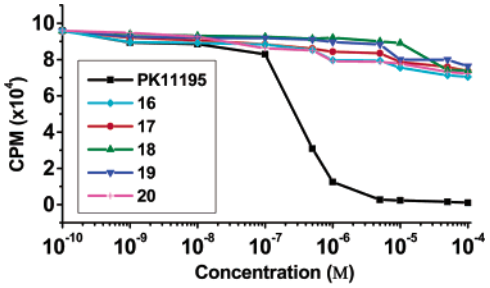


Figure 7. Displacement of ^{3}H-PK11195 by PK11195 and benzobacteriochlorins **16**−**20** atvariable concentrations. Compounds **16**−**20** produce limited displacement of ^{3}H-PK11195 indicating their limited peripheral benzodiazepine receptor binding ability.

Table 1. HSA Site II Binding Abilities of Various Benzobacteriochlorins

benzobacteriochlorin	**16**	**17**	**18**	**19**	**20**
K ($\times 10^7$ M^{-1})[a]	1.06	1.75	1.44	1.61	1.60

[a] The binding constant values of benzobacteriochlorins to HSA (Site II).

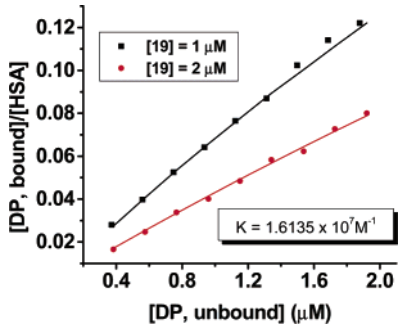


Figure 8. Langmuir plot of binding between DP and HSA Site II with benzobacteriochlorin **19**. Concentration of HSA was kept constant (1.0 μM). Bacteriochlorin **19** was evaluated at 1.0 and 2.0 μM concentrations. Concentrations of DP was varied from 0 to 2.0 μM. Data were simulated with a competitive binding model. Nonlinear least-squares curve fittings were performed with Origin 5.0 (Microcal Software Inc.) on IBM-PC computer.

previously.[32] The following formula was derived from a competitive binding model that was used for this study:

$$r_A = 2K_A A_u/\{1 + K_A A_u + K_B B_t - K_B P_t \pm [(1 + K_A A_u + K_B P_t - K_B B_t)^2 + 4K_B B_t + 4K_A K_B A_u B_t]^{1/2}\}$$

where r_A is the number of probes binding with each HSA molecule. K_A is the binding constant of the probe (DP). K_B is the binding constant of the drug (benzobacteriochlorins **16**−**20**). A_u is the concentration of unbound probe. B_t is the total concentration of the drug. P_t is the total concentration of the HSA. The data analysis was performed on an IBM-PC with Origin (version 5.0 for Windows, Microcal Software Inc.). Nonlinear least-squares curve fittings method was used for the calculation of K_B.

Conclusion

In summary, starting from chlorophyll-a, an easily available natural product, a series of stable benzobacteriochlorins **16**−**20** with long wavelength absorption near 737−805 nm were synthesized in moderate overall yield. In preliminary in vitro and in vivo studies, among the bacteriochlorins investigated, benzobacteriochlorin **19** was found to be most effective and showed reduced skin phototoxicity compared to Photofrin at their respective therapeutic doses. Similarly to other known porphyrin-based effective photosensitizers, benzochlorins **16**−**20** also were found to localize in mitochondria and exhibited competitive binding to site II of HSA (a peripheral diazepine-binding site). No direct correlation between the HSA (Site II) binding constant values of various photosensitizers with photosensitizing efficacy was observed. However, this technique could be used as a simple in vitro screening tool in selecting the compounds for in vivo studies. The detailed biological evaluation (pharmacokinetic and pharmacodynamic characteristics) of benzobacteriochlorin **19** and a series of the related *N*-substituted analogues with variable lipophilicity are currently in progress.

Experimental Section

^{1}H and ^{19}F NMR spectra were recorded in $CDCl_3$ solutions at 400 MHz Brucker instrument. Chemical shifts are reported in ppm with $CDCl_3$ as internal standard (for ^{1}H, 7.26 ppm) and TFA as external standard (for ^{19}F, 0.00 ppm). Proton peak assignments were based on 2D NMR (H−H COSY and/or ROESY) analysis. UV−vis spectra were recorded on a Varian (Cary-50 Bio) spectrophotometer. Column chromatographic separations were performed over silica gel 60 (70−230 mesh) or neutral alumina (Brockmann grade III, ~150 mesh). Preparative TLC was performed on silica 20 × 20 cm TLC plates (Analtech). Methylpheophorbide *a*, the starting material used for the preparation of a series of desired benzobacteriochlorin analogues was isolated from *Spirulina pacifica* by following the literature procedure.

Methyl Mesopyropheophorbide *a* (1b). A solution of Zn(OAc)$_2$·2H$_2$O (1.11 g) in methanol (45 mL) was added to a solution of methyl pyropheophorbide *a* **1a** (1.11 g) in dichloromethane (75 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was then washed with water (4 × 100 mL), and the organic layer was collected and dried over Na_2SO_4. Solvent was removed with a rotavapor at reduced pressure, and the residue was dissolved in THF (100 mL). Et_3N (0.2 mL) and Pd/C (10%, 100 mg) were then added to above solution. The resultant mixture was hydrogenated (with a hydrogen balloon) at room temperature for 15 h and then filtered through a pad of Celite. The solvent was removed with a rotavapor at reduced pressure, and the residue was treated with TFA (30 mL) for 1 h at room temperature. The reaction mixture was poured into ice and extracted with CH_2Cl_2 until the water layer was clear. The CH_2Cl_2 layers were combined and washed with water (2 × 200 mL) and 5% $NaHCO_3$ (1 × 200 mL). The CH_2Cl_2 layer was collected and dried over Na_2SO_4. The solvent was removed after filtration, and the residue was purified by column chromatography over alumina eluted with CH_2Cl_2/EtOAc (v/v 10/1). The title compound (1.09 g) was obtained with an overall yield of 98%.

Mesochlorin e_6 Trimethyl Ester (2b). Following the procedure described for the preparation of **1b**, the title compound was obtained in 96% yield from chlorin **2a**.

Mesochlorin p_6 Trimethyl Ester (3b). Starting from **3a** and following the procedure described for the preparation of **1b**, the title compound was obtained in 92% yield from compound **3a**. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 398 (150305), 496 (11402), 527 (4164), 605 (5453), 656 (40749). ^{1}H NMR δ 9.68 (1H, s, *meso* H), 9.31 (1H, s, *meso* H), 8.57 (1H, s, *meso* H), 5.15 (1H, dd, J = 8.8, 2.4 Hz, H-17), 4.37 (1H, q, J = 7.3 Hz, H-18), 4.22, 4.16, 3.63, 3.52, 3.29, 3.25 (each 3H, s, CH_3-2, CH_3-7, CH_3-12 and 3 × methyl esters), 3.82 (2H, q, J = 7.6 Hz,

CH_3CH_2-3 or CH_3CH_2-8), 3.74 (2H, q, J = 7.6 Hz, CH_3CH_2-3 or CH_3CH_2-8), 2.36, 2.20, 2.04, 1.87 (each 1H, m, CH_3-OOCCH_2CH_2-17), 1.85 (3H, d, J = 7.4 Hz, CH_3-18), 1.73 (3H, t, J = 7.5 Hz, CH_3CH_2-3 or CH_3CH_2-8), 1.70 (3H, t, J = 7.5 Hz, CH_3CH_2-3 or CH_3CH_2-8), −0.84 (2H, br, 2 × NH). MS (ESI) m/z 649.3 ([M + Na]$^+$, 100). Anal. ($C_{36}H_{42}N_4O_6$) C, H, N.

Mesopurpurin-18-*N*-hexylimide Methyl Ester (4b). Starting from **4a** and following the procedure described for the preparation of **1b**, the title compound was obtained in 95% yield. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 364 (35629), 417 (117252), 508 (6640), 554 (14738), 638 (6154), 694 (33848). ^{1}H NMR δ 9.62 (1H, s, *meso* H), 9.22 (1H, s, *meso* H), 8.51 (1H, s, *meso* H), 5.39 (1H, dd, J = 8.3, 2.4 Hz, H-17), 4.46 [2H, m, CH_3-$(CH_2)_4CH_2$N], 4.33 (1H, q, J = 7.3 Hz, H-18), 3.84, 3.56, 3.25, 3.20 (each 3H, s, CH_3-2, CH_3-7, CH_3-12 and methyl ester), 3.77 (2H, q, J = 7.7 Hz, CH_3CH_2-3 or CH_3CH_2-8), 3.67 (2H, q, J = 7.7 Hz, CH_3CH_2-3 or CH_3CH_2-8), 2.67, 2.35, 1.99 [1H, 2H, 3H, m, CH_3OOCCH_2CH_2-17 and $CH_3(CH_2)_3CH_2CH_2$N], 1.75 (3H, d, J = 7.3 Hz, CH_3-18), 1.71 (3H, t, J = 7.6 Hz, CH_3CH_2-3 or CH_3CH_2-8), 1.68 (3H, t, J = 7.6 Hz, CH_3CH_2-3 or CH_3CH_2-8), 1.62 (2H, m, $CH_3CH_2CH_2CH_2CH_2CH_2$N), 1.45 (4H, m, CH_3-$CH_2CH_2CH_2CH_2CH_2$N), 0.95 [3H, t, J = 7.2 Hz, $CH_3(CH_2)_3$-CH_2CH_2N], 0.02, −0.15 (each 1H, br, 2 × NH). MS (ESI) m/z 664.5 ([M + 1]$^+$, 100). Anal. ($C_{40}H_{49}N_5O_4$·0.5H_2O) C, H, N.

Mesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide Methyl Ester (5b). Starting from **5a** and following the procedure described for the preparation of **1b**, the title compound was obtained in 92% yield. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 362 (54373), 417 (174414), 508 (9263), 545 (26734), 641 (10167), 696 (53996). ^{1}H NMR δ 9.54 (1H, s, H-10), 9.15 (1H, s, H-5), 8.48 (1H, s, H-20), 8.24 (2H, s, 2 × CH at position 2 and 6 on the benzene ring), 7.81 (1H, s, CH at position 4 on the benzene ring), 5.77 (2H, s, CH_2 of benzyl), 5.33 (1H, m, H-17), 4.33 (1H, q, J = 7.5 Hz, H-18), 3.79, 3.56, 3.23, 3.15 (each 3H, s, CH_3-2, CH_3-7, CH_3-12 and 1 × methyl ester), 3.74 (2H, q, J = 7.7 Hz, CH_3CH_2-3 or CH_3CH_2-8), 3.61 (2H, q, J = 7.7 Hz, CH_3CH_2-3 or CH_3CH_2-8), 2.69, 2.38, 1.95 (1H, 2H, 1H, m, CH_3OOCCH_2CH_2-17), 1.77 (3H, d, J = 7.2 Hz, CH_3-18), 1.70 (3H, t, J = 7.6 Hz, CH_3CH_2-3 or CH_3CH_2-8), 1.66 (3H, t, J = 7.6 Hz, CH_3CH_2-3 or CH_3CH_2-8), 0.25 (1H, br, *NH*), 0.04 (1H, br, *NH*). MS (ESI) m/z 828.4 ([M + Na]$^+$, 100). Anal. ($C_{43}H_{41}F_6N_5O_4$·2H_2O) C, H, N.

***vic*-7,8-Dihydroxymethylmesopyropheophorbide *a* (6).**[13,15] Pyridine (1.0 mL) and a solution of OsO_4 (1.0 g) in Et_2O (10 mL) were successively added to a solution of methyl mesopyropheophorbide *a* (**1b**) (1.06 g) in dry CH_2Cl_2 (120 mL). The mixture was stirred at room temperature for 24 h. H_2S gas was bubbled into the reaction mixture for 5 min to decompose the unreacted OsO_4. Nitrogen was then bubbled into above mixture to remove H_2S. The mixture was filtered through a pad of Celite. The filtrate was evaporated, and the residue was purified by column chromatography over silica gel eluted with CH_2Cl_2/EtOAc (first v/v 10/1, then 5/1). The title compound (735 mg) was obtained in 65% yield and the unreacted starting material **1b** (54 mg) was also recovered.

***vic*-7,8-Dihydroxymesochlorin e_6 Trimethyl Ester (7).**[13,16] Mesochlorin e_6 trimethyl ester **2b** (310 mg) was reacted with OsO_4 (350 mg) by following the procedure described for the preparation of **6**, and the title compound was obtained in 52% yield (182 mg). The unreacted **2b** (35 mg) was also recovered.

***vic*-7,8-Dihydroxymesochlorin p_6 Trimethyl Ester (8).** Mesochlorin p_6 trimethyl ester **3b** (367 mg) was reacted with OsO_4 (500 mg) by following the procedure described for the preparation of **6** and the title compound (342 mg) was obtained as a mixture of two isomers (2.4:1) in 83% yield. The unreacted **3b** (21 mg) was also recovered. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 354 (97147), 382 (74713), 450 (3505), 480 (5558), 511 (21382), 723 (25789). ^{1}H NMR δ 8.66, 8.65 (1H, s, *meso* H), 8.45 (1H, s, *meso* H), 8.22, 8.19 (1H, s, *meso* H), 4.92, 4.86 (1H, m, H-17), 4.11 (1H, m, H-18), 4.14, 4.10, 3.54, 3.37, 3.14 (each 3H, splitting s, CH_3-2, CH_3-12 and 3 × methyl esters), 3.61 (2H, q, J = 7.9 Hz, CH_3CH_2-3), 2.48 (2H, q, J = 7.3 Hz, CH_3CH_2-8), 2.32, 2.13, 2.04, 1.79 (total 4H, m, CH_3OOCCH_2CH_2-17), 1.99, 1.91 (3H, s, CH_3-7), 1.77, 1.74 (3H, d, J = 7.5 Hz, CH_3-18), 1.63 (3H, t, J = 7.5 Hz, CH_3CH_2-3), 1.18, 1.12 (3H, t, J = 7.6 Hz, CH_3CH_2-8), 0.17, 0.07, −0.09, −0.19 (2H, br, 2 × NH). MS (ESI) m/z 683.4 ([M + Na]$^+$, 100). Anal. ($C_{36}H_{44}N_4O_8$·1.5H_2O) C, H, N.

***vic*-7,8-Dihydroxymesopurpurin-18-*N*-hexylimide Methyl Ester (9).** Mesopurpurin-18-*N*-hexylimide methyl ester **4b** (578 mg) was reacted OsO_4 (500 mg) by following the procedure described for the preparation of **6**, the title compound (519 mg) was obtained as a mixture of two isomers (1:1) in 85% yield. The unreacted **4b** (80 mg) was also recovered. A small amount of the isomer mixture was separated with preparative silica TLC plates using CH_2Cl_2/EtOAc (v/v 5/1) as developing solvent. **The faster moving isomer**: UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 367 (112349), 411 (47574), 536 (42561), 757 (31650). ^{1}H NMR δ 8.67 (1H, s, *meso* H), 8.49 (1H, s, *meso* H), 8.21 (1H, s, *meso* H), 5.14 (1H, br d, J = 8.5 Hz, H-17), 4.11 [3H, m, H-18 and $CH_3(CH_2)_4CH_2$N], 3.89 (1H, s, OH), 3.60 (2H, q, J = 7.7 Hz, CH_3CH_2-3), 3.55, 3.54, 3.13 (each 3H, s, CH_3-2, CH_3-12 and CH_3OOCCH_2CH_2-17), 2.92 (1H, s, OH), 2.58 (3H, m, CH_3-OOCCH_2CH_2-17 and one proton of CH_3OOCCH_2CH_2-17), 2.28 (2H, q, J = 10.1 Hz, CH_3CH_2-8), 1.89 (4H, s, CH_3-7 and one proton of CH_3OOCCH_2CH_2-17), 1.83 (2H, m, $CH_3CH_2CH_2$-$CH_2CH_2CH_2$N), 1.68 (3H, d, J = 7.3 Hz, CH_3-18), 1.63 (3H, t, J = 7.7 Hz, CH_3CH_2-3), 1.50 (2H, m, $CH_3CH_2CH_2CH_2CH_2$-CH_2N), 1.39 (4H, m, $CH_3CH_2CH_2CH_2CH_2CH_2$N), 1.28 (3H, t, J = 7.6 Hz, CH_3CH_2-8), 0.92 (3H, t, J = 7.1 Hz, $CH_3CH_2CH_2$-$CH_2CH_2CH_2$N), 0.47 (1H, s, NH), 0.11 (1H, s, NH). MS (ESI) m/z 720.4 ([M + Na]$^+$, 100). **The slower moving isomer**: UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 368 (99597), 412 (42859), 536 (38058), 762 (26798). ^{1}H NMR δ 8.71 (1H, s, *meso* H), 8.47 (1H, s, *meso* H), 8.24 (1H, s, *meso* H), 5.21 (1H, dd, J = 8.8, 2.3 Hz, H-17), 4.38 [2H, m, $CH_3(CH_2)_4CH_2$N], 4.17 (1H, q, J = 7.6 Hz, H-18), 3.60 (4H, q, J = 7.6 Hz, CH_3CH_2-3), 3.58, 3.57, 3.13 (each 3H, s, CH_3-2, CH_3-12 and CH_3OOCCH_2CH_2-17), 3.58 (1H, s, OH, overlapped with ring methyl or methyl of methyl ester), 2.98 (1H, s, OH), 2.65 (1H, m, one proton of CH_3-OOCCH_2CH_2-17), 2.49 (2H, q, J = 7.7 Hz, CH_3CH_2-8), 2.34 (2H, m, CH_3OOCCH_2CH_2-17), 1.95 (2H, s, CH_3-7), 1.94 (3H, m, $CH_3CH_2CH_2CH_2CH_2CH_2$N and one proton of CH_3OOCCH_2-CH_2-17), 1.66 (3H, d, J = 7.4 Hz, CH_3-18), 1.62 (3H, t, J = 7.7 Hz, CH_3CH_2-3), 1.56 (2H, m, $CH_3CH_2CH_2CH_2CH_2CH_2$N), 1.43 (4H, m, $CH_3CH_2CH_2CH_2CH_2CH_2$N), 1.17 (3H, t, J = 7.2 Hz, CH_3CH_2-8), 0.93 (3H, t, J = 7.3 Hz, $CH_3CH_2CH_2CH_2CH_2$-CH_2N), 0.44 (1H, s, *NH*), 0.02 (1H, s, *NH*). MS (ESI) m/z 720.4 ([M + Na]$^+$, 100). Anal. ($C_{40}H_{51}N_5O_6$) C, H, N.

***vic*-7,8-Dihydroxymesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide Methyl Ester (10).** Mesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide methyl ester **5b** (340 mg) was reacted with OsO_4 (500 mg) by following the procedure described for the preparation of **6**, and the title compound (263 mg) was obtained as a mixture of two isomers (1:1) in 74% yield. The unreacted **5b** (20 mg) was also recovered. A small amount of the isomer mixture was separated with preparative silica TLC plates using CH_2Cl_2/EtOAc (v/v 5/1) as developing solvent. **The faster moving isomer**: UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 368 (107382), 413 (45711), 540 (44541), 756 (29415). ^{1}H NMR δ 8.64 (1H, s, meso H), 8.49 (1H, s, meso H), 8.19 (1H, s, meso H), 7.91 (2H, s, 2 × CH at position 2 and 6 on benzene ring), 7.72 (1H, s, 1 × CH at position 4 on benzene ring), 5.04 (1H, dd, J = 8.8, 2.5 Hz, H-17), 4.95 (2H, m, CH_2 of benzyl), 4.10 (1H, q, J = 7.4 Hz, H-18), 3.59 (2H, q, J = 7.6 Hz, CH_3CH_2-3), 3.50, 3.48, 3.12 (each 3H, s, CH_3-2, CH_3-12 and CH_3OOCCH_2CH_2-17), 2.54, 2.28, 2.14, 1.79 (3H, 1H, 1H, 1H, m, CH_3OOCCH_2CH_2-17 and CH_3CH_2-8), 1.91 (1H, s, CH_3-7), 1.73 (3H, d, J = 7.2 Hz, CH_3-18), 1.63 (3H, t, J = 7.5 Hz, CH_3CH_2-3), 1.26 (3H, t, J = 7.2 Hz, CH_3CH_2-8), 0.68 (1H, s, NH), 0.36 (1H, s, NH). MS (ESI) m/z 862.4 ([M + Na]$^+$, 100). Anal. ($C_{43}H_{43}F_6N_5O_6$) C, H, N. **The slower moving isomer**: UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 368 (107261), 414 (46658), 540 (44895), 764 (27995). ^{1}H NMR δ 8.65 (1H, s, *meso* H), 8.44 (1H, s, *meso* H), 8.20 (1H, s, *meso* H), 8.14 (2H, s, 2 × CH at position 2 and 6 on benzene ring), 7.78 (1H, s, 1 × CH at position 4 on benzene ring), 5.62 (2H, m, CH_2 of benzyl), 5.13 (1H, dd, J = 8.9, 2.9 Hz, H-17), 4.16 (1H, q, J = 7.2 Hz, H-18),

3.54 (2H, q, CH_3CH_2-3, overlapped with ring methyls or methyl of methyl ester), 3.56, 3.52, 3.07 (each 3H, s, CH_3-2, CH_3-12 and $CH_3OOCCH_2CH_2$-17), 2.64, 2.37, 2.28, 1.89 (1H, 1H, 1H, 1H, m, $CH_3OOCCH_2CH_2$-17), 2.48 (2H, m, CH_3CH_2-8), 1.93 (1H, s, CH_3-7), 1.66 (3H, d, J = 7.3 Hz, CH_3-18), 1.61 (3H, t, J = 7.6 Hz, CH_3CH_2-3), 1.16 (3H, t, J = 7.3 Hz, CH_3CH_2-8), 0.73 (1H, s, NH), 0.29 (1H, s, NH). MS (ESI) m/z 862.4 ($[M + Na]^+$, 100). Anal. ($C_{43}H_{43}F_6N_5O_6$) C, H, N.

8-Vinylmethylmesopyropheophorbide a (11). *vic*-7,8-Dihydroxymethylmesopyropheo phorbide *a* **6** (364 mg) was dissolved in *o*-dichlorobenzene (30 mL). The solution was refluxed for 1.5 h under an atmosphere of N_2. After being cooled to room temperature, the reaction mixture was loaded on a short silica column and eluted with hexanes to remove *o*-dichlorobenzene and then 10% MeOH/CH_2Cl_2 to give a mixture. The mixture was further purified with silica gel column eluted with 3% MeOH/CH_2Cl_2. The title compound (184 mg) was obtained in 54% yield. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 418 (143057), 509 (11678), 541 (7213), 602 (9703), 657 (53238). ^{1}H NMR δ 9.22 (1H, s, H-10), 9.04 (1H, s, H-5), 8.47 (1H, s, H-20), 7.66 (1H, dd, J = 17.9, 11.8 Hz, H-8^1), 5.99 (1H, d, J = 17.4 Hz, H-8^2 trans), 5.88 (1H, d, J = 9.7 Hz, H-8^2 cis), 5.13 (2H, dd, AB system, J = 20.2 Hz, $-COCH_2$-15), 4.46 (1H, dq, J = 7.7, 2.2 Hz, H-18), 4.25 (1H, m, H-17), 3.73 (2H, q, J = 7.6 Hz, CH_3CH_2-3), 3.66 (3H, s, $CH_3OOCCH_2CH_2$-17), 3.40 (3H, s, CH_3-12), 3.29 (3H, s, CH_3-2), 3.20 (3H, s, CH_3-7), 2.68 (1H, m, one proton of $CH_3OOCCH_2CH_2$-17), 2.58 (1H, m, one proton of $CH_3OOCCH_2CH_2$-17), 2.29 (2H, m, one proton of CH_3-$OOCCH_2CH_2$-17 and one proton of $CH_3OOCCH_2CH_2$-17), 1.85 (3H, d, J = 7.0 Hz, CH_3-18), 1.71 (3H, t, J = 7.6 Hz, CH_3CH_2-3), 0.16 (1H, s, NH), −1.86 (1H, s, NH). MS (FAB) m/z 548.2 (M^+, 100). HRMS (FAB): Calcd for $C_{43}H_{36}N_4O_3$, 548.2787; Found 548.2763. Anal. ($C_{34}H_{36}N_4O_3 \cdot 0.5H_2O$) C, H, N.

8-Vinylmesochlorin e_6 Trimethyl Ester (12). A solution of *vic*-7,8-Dihydroxymesochlorin e_6 trimethyl ester 7 (491 mg) in *o*-dichlorobenzene (40 mL) was refluxed for 1.5 h. After workup (following the procedure described for the preparation of **11**), the resultant mixture was purified by column chromatography over silica gel eluted with CH_2Cl_2/acetone (v/v 15/1). The title compound was obtained in 41% yield (192 mg). UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 405 (145190), 501 (11460), 597 (4878), 651 (35620). ^{1}H NMR δ 9.84 (1H, s, H-10), 9.41 (1H, s, H-5), 8.68 (1H, s, H-20), 8.03 (1H, dd, J = 18.0, 11.0 Hz, H-8^1), 6.13 (1H, dd, J = 17.0, 2.4 Hz, H-8^2 trans), 6.00 (1H, J = 11.9, 1.9 Hz, H-8^2 cis), 5.31 (2H, dd, AB system, J = 18.0 Hz, CH_3-$OOCCH_2$-15), 4.48 (1H, q, J = 7.4 Hz, H-18), 4.41 (1H, dd, J = 9.8, 2.1 Hz, H-17), 3.86 (2H, q, J = 7.6 Hz, CH_3CH_2-3), 4.28, 3.80, 3.66, 3.58, 3.42, 3.35 (each 3H, s, CH_3-2, CH_3-7, CH_3-12, and 3 × methyl esters), 2.58, 2.23, 1.79 (1H, 2H, 1H, m, CH_3-$OOCCH_2CH_2$-17), 1.77 (3H, d, J = 6.9 Hz, CH_3-18), 1.76 (3H, t, J = 8.3 Hz, CH_3CH_2-3), −1.27 (1H, br, NH), −1.30 (1H, br, NH). MS (FAB) m/z 639.4 (MH^+, 100). HRMS (FAB): Calcd for $C_{37}H_{43}N_4O_6$ [M + H], 639.3182; Found 639.3168. Anal. ($C_{37}H_{42}N_4O_6$) C, H, N.

8-Vinylmesochlorin p_6 Trimethyl Ester (13). A solution of *vic*-7,8-Dihydroxymesochlorin p_6 trimethyl ester 8 (342 mg) in *o*-dichlorobenzene (30 mL) was refluxed for 1.5 h. After standard workup (following the procedure described for the preparation of **11**), the resultant mixture was purified by column chromatography over silica gel eluted with CH_2Cl_2/EtOAc (v/v 15/1). The title compound (128 mg) was obtained in 40% yield. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 405 (142 001), 502 (11 009), 601 (4725), 655 (35 171). ^{1}H NMR δ 9.84 (1H, s, H-10), 9.35 (1H, s, H-5), 8.58 (1H, s, H-20), 7.97 (1H, dd, J = 18.1, 10.9 Hz, H-8^1), 6.10 (1H, d, J = 18.8 Hz, H-8^2 trans), 6.00 (1H, J = 12.0, H-8^2 cis), 5.14 (1H, dd, J = 9.8, 2.2 Hz, H-17), 4.38 (1H, q, J = 7.4 Hz, H-18), 3.83 (2H, q, J = 7.6 Hz, CH_3CH_2-3), 4.22, 4.16, 3.62, 3.53, 3.37, 3.30 (each 3H, s, CH_3-2, CH_3-7, CH_3-12, and 3 × methyl esters), 2.39, 2.21, 2.08, 1.86 (each 1H, m, $CH_3OOCCH_2CH_2$-17), 1.85 (3H, d, J = 6.5 Hz, CH_3-18), 1.74 (3H, t, J = 7.6 Hz, CH_3CH_2-3), −0.84 (2H, br, 2 × -NH). MS (FAB) m/z 624.4 (M^+, 100). HRMS (FAB): Calcd for $C_{36}H_{40}N_4O_6$, 624.2948; Found 624.2956. Anal. ($C_{36}H_{40}N_4O_6 \cdot 0.5H_2O$) C, H, N.

8-Vinylmesopmesourpurin-18-*N*-hexylimide Methyl Ester (14). *vic*-7,8-Dihydroxymesopurpurin-18-*N*-hexylimide methyl ester **9** (519 mg) in *o*-dichlorobenzene (50 mL) was refluxed for 1.5 h. After the standard workup by following the procedure described for the preparation of **11**, the resultant mixture was purified by column chromatography over silica gel eluted with CH_2Cl_2/EtOAc (v/v 20/1). The title compound was obtained in 41% (201 mg) yield. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 367 (35101), 422 (116543), 512 (8313), 547 (11085), 637 (5542), 690 (32946). ^{1}H NMR δ 9.75 (1H, s, H-10), 9.25 (1H, s, H-5), 8.51 (1H, s, H-20), 7.87 (1H, dd, J = 18.0, 11.9 Hz, H-8^1), 6.08 (1H, d, J = 17.9 Hz, H-8^2 trans), 5.98 (1H, d, J = 11.6 Hz, H-8^2 cis), 5.39 (1H, dd, J = 8.8, 2.2 Hz, H-17), 4.45 (2H, m, $NCH_2CH_2CH_2$-$CH_2CH_2CH_3$), 4.34 (1H, q, J = 7.2 Hz, H-18), 3.77 (2H, q, J = 8.0 Hz, CH_3CH_2-3), 3.80, 3.56, 3.30, 3.25 (each 3H, s, CH_3-2, CH_3-7, CH_3-12, and 1 × methyl esters), 2.68, 2.38, 2.11 (1H, 2H, 1H, m, $CH_3OOCCH_2CH_2$-17), 1.98 (2H, m, $NCH_2CH_2CH_2$-$CH_2CH_2CH_3$), 1.76 (3H, d, J = 7.2 Hz, CH_3-18), 1.71 (3H, t, J = 7.7 Hz, CH_3CH_2-3), 1.60 (2H, m, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 1.44 (4H, m, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 0.95 (3H, t, J = 7.0 Hz, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 0.02 (1H, br, NH), −0.15 (1H, br, NH). MS (FAB) m/z 662.5 (MH^+, 100). HRMS (FAB): Calcd for $C_{40}H_{48}N_5O_4$, 662.3706 (M + H); Found 662.3685. Anal. ($C_{40}H_{47}N_5O_4$) C, H, N.

8-Vinylmesopurpurin-18-*N*-3, 5-bis(trifluoromethyl)-benzylimide Methyl Ester (15). *vic*-7,8-Dihydroxymesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide methyl ester **10** (243 mg) in *o*-dichlorobenzene (30 mL) was refluxed for 1.5 h. After workup by following the procedure for the preparation of **11**, the resultant mixture was purified by column chromatography over silica gel eluted with CH_2Cl_2/acetone (v/v 99/1). The title compound was obtained in 51% (120 mg) yield. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 367 (45 775), 422 (152 778), 513 (10 230), 548 (17 650), 638 (8207), 693 (45 418). ^{1}H NMR δ 9.71 (1H, s, H-10), 9.21 (1H, s, H-5), 8.48 (1H, s, H-20), 8.23 (2H, s, 2 × CH at position 2 and 6 on benzene ring), 7.84 (1H, dd, J = 18.0, 10.7 Hz, H-8^1), 7.80 (1H, s, CH at position 4 on benzene ring), 6.07 (1H, d, J = 17.7 Hz, H-8^2 trans), 5.98 (1H, d, J = 11.8 Hz, H-8^2 cis), 5.76 (2H, s, CH_2 of benzyl), 5.32 (1H, m, H-17), 4.33 (1H, q, J = 7.3 Hz, H-18), 3.78, 3.55, 3.27, 3.23 (each 3H, s, CH_3-2, CH_3-7, CH_3-12, and 1 × methyl ester), 3.75 (2H, q, J = 7.6 Hz, CH_3CH_2-3), 2.68, 2.38, 1.94 (1H, 2H, 1H, m, $CH_3OOCCH_2CH_2$-17), 1.76 (3H, d, J = 7.1 Hz, CH_3-18), 1.71 (3H, t, J = 7.6 Hz, CH_3CH_2-3), 0.26 (1H, br, NH), 0.05 (1H, br, NH). MS (ESI) m/z 804.5 (MH^+, 100). HRMS (FAB): Calcd for $C_{43}H_{40}F_6N_5O_4$, 804.2985 (M + H); Found 804.2983. Anal. ($C_{43}H_{39}F_6N_5O_4$) C, H, N.

Benzobacteriochlorins 16. DMAD (2.0 mL) was added to a solution of 8-vinylmethylmesopyropheophorbide *a* **11** (95 mg) in toluene (20 mL). The mixture was refluxed for 5 h under nitrogen. After the mixture was cooled to room temperature, another portion of DMAD (1.5 mL) was added, and the solution was further refluxed for 2.5 h. The solvent and excess DMAD were removed with rotavapor. The residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 8/1) as developing solvent. The intermediate benzobacteriochlorin (18 mg) and the unreacted **11** (55 mg) were isolated. The intermediate compound (18 mg) was dissolved in CH_2Cl_2 (30 mL), and 2 drops of DBU was added. The resulting solution was stirred at room temperature for 5 min. The solvent was removed, and the residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 8/1) as developing solvent. The title compound (18 mg) was obtained as a mixture of two isomers (1:1) in 36% converted yield in two steps. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 334 (38 860), 402 (36 471), 445 (28 623), 476 (40 414), 563 (9440), 666 (9705), 737 (21 686). ^{1}H NMR δ 8.72 (1H, s, H-10), 8.11 (1H, s, H-5), 7.98, 7.97 (each 0.5H, s, H-20), 7.69 (1H, d, J = 6.0 Hz, H-8^2), 7.09 (1H, d, J = 6.3 Hz, H-8^1), 4.93, 4.92, 4.77 (0.5H, 0.5H, 1H, two sets of dd, AB system, J = 20.0 Hz, $-COCH_2$-15), 4.78, 4.77 (each 0.5H, s, H-8^4), 4.14 (1H, q, J = 7.4 Hz, H-18), 3.97 (1H, m, H-17), 3.95 (3H, s, CH_3-OOC-8^3), 3.64, 3.63 (each 1.5H, s, $CH_3OOCCH_2CH_2$-17), 3.57 (2H, q, J = 7.7 Hz, CH_3CH_2-3), 3.37, 3.34 (each 1.5H, s, CH_3-12), 3.14, 3.13 (each 1.5H, s, CH_3OOC-8^4), 3.08 (3H, s, CH_3-2),

2.51, 2.24 (each 2H, m, $CH_3OOCCH_2CH_2$-17), 1.69 (3H, s, CH_3-7), 1.68 (3H, splitting d, CH_3-18), 1.63 (3H, t, $J = 7.6$ Hz, CH_3CH_2-3), 0.10, 0.08 (each 1H, br s, 2 × NH). MS (FAB) *m/z* 690.2 (M^+, 100). HRMS (FAB): Calcd for $C_{40}H_{42}N_4O_7$, 690.3053; Found 690.3062.

Benzobacteriochlorins 17. The intermediate benzobacteriochlorin **21** (15 mg) was dissolved in CH_2Cl_2 (30 mL), and 2 drops of DBU was added to above solution. The resultant solution was stirred at room temperature for 5 min. The solvent was removed, and the residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 8/1) as developing solvent. The title compound (15 mg) was obtained as a mixture of four isomers (1:0.8:0.8:0.5) in quantitative yield. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 330 (42 854), 399 (44 598), 470 (44 965), 570 (9911), 673 (8351), 744 (32 393). ^{1}H NMR δ 9.07, 9.05, 9.00, 8.97, 8.70, 8.57, 8.38, 8.37, 8.35, 8.34, 8.27, 8.25 (3H, each s, 3 × *meso* H), 7.73, 7.66 (1H, m, H-8^2), 7.16 (1H, m, H-8^1), 5.08 (2H, m, CH_3OOCCH_2-15), 4.84, 4.81, 4.71, 4.61 (1H, s, s, d, d, H-8^4), 4.20, 4.19, 4.18, 3.95, 3.91, 3.89, 3.77, 3.75, 3.74, 3.73, 3.64, 3.63, 3.36, 3.36, 3.32, 3.22, 3.21, 3.18, 3.17, 3.09, 3.05 (21H, each s, 5 × methyl esters, CH_3-2 and CH_3-12), 4.15 (2H, m, H-17 and H-18), 3.66 (2H, m, CH_3CH_2-3), 2.51, 2.17, 1.72 (1H, 2H, 1H, m, $CH_3OOCCH_2CH_2$-17), 1.80−1.50 (9H, m, CH_3-7, CH_3CH_2-3 and CH_3-18), −0.08, −0.10, −0.37, −0.40, −0.46, −0.48, −0.74, −0.77 (2H, 2 × NH). MS (FAB) *m/z* 780.2 (M^+, 100). HRMS (FAB): Calcd for $C_{43}H_{48}N_4O_{10}$, 780.3370; Found 780.3394.

Benzobacteriochlorins 18. DMAD (2.0 mL) was added to a solution of 8-vinylmesochlorin p_6 trimethyl ester **13** (54 mg) in toluene (15 mL). The mixture was refluxed for 5 h under N_2. The solvent and excess DMAD were removed with rotavapor. The residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 15/1) as developing solvent. The intermediate benzobacteriachlorin (crude) along with the unreacted **13** (12 mg) was also recovered. The above intermediate compound was dissolved in CH_2Cl_2 (30 mL), and 2 drops of DBU was added to above solution. The resultant solution was stirred at room temperature for 5 min. The solvent was removed, and the residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 15/1) as developing solvent. The title compound (13 mg) was obtained as a mixture of two isomers (2:1) in 26% converted yield in two steps. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 402 (38 000), 474 (30 000), 573 (8200), 687 (7400), 759 (20 000). ^{1}H NMR δ 9.02, 8.98 (1H, each s, *meso* H), 8.33 (1H, splitting s, *meso* H), 8.21, 8.20 (1H, each s, *meso* H), 7.71, 7.70 (1H, each d, $J = 5.4$ Hz, $J = 5.9$ Hz, H-8^2), 7.15, 7.10 (1H, each d, $J = 5.4$ Hz, $J = 6.1$ Hz, H-8^1), 4.91, 4.89 (1H, m, H-17), 4.80, 4.78, 4.73, 4.53 (1H, each s, H-8^4), 4.15, 4.09, 3.95, 3.54, 3.40, 3.14, 3.10 (21H, each splitting s, 5 × methyl esters, CH_3-2 and CH_3-12), 4.13 (1H, m, H-18), 3.61 (2H, q, $J = 7.5$ Hz, CH_3CH_2-3), 2.33, 2.08, 1.79 (1H, 2H, 1H, m, $CH_3OOCCH_2CH_2$-17), 1.75 (3H, splitting d, CH_3-18), 1.66 (3H, s, CH_3-7), 1.64 (3H, t, $J = 7.6$ Hz, CH_3CH_2-3), 0.18, −0.15 (each 1H, br, 2 × NH). MS (FAB) *m/z* 766.2 (M^+, 100). HRMS (FAB): Calcd for $C_{42}H_{46}N_4O_{10}$, 766.3214; Found 766.3228.

Benzobacteriochlorins 19. DMAD (2.0 mL) was added to a solution of 8-Vinylmesopurpurin-18-*N*-hexylimide methyl ester **14** (95 mg) in toluene (20 mL). The mixture was refluxed for 5 h under N_2. The solvent and excess DMAD were removed with rotavapor. The residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 50/1) as developing solvent. The intermediate benzobacteriochlorin (55 mg) was obtained and a small amount of the unreacted **14** was recovered. The intermediate product was dissolved in CH_2Cl_2 (30 mL), 2 drops of DBU was added, and the resulting solution was stirred at room temperature for 5 min. The solvent was removed, and the residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 50/1) as developing solvent. The title compound (18 mg) was obtained as a mixture of four isomers in 37% converted yield in two steps. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 341 (36740), 383 (39820), 436 (70 620), 483 (35 640), 668 (6820), 714 (9900), 801 (29 810). ^{1}H NMR δ 9.11, 9.10, 9.03, 8.69, 8.64, 8.34, 8.33, 8.31, 8.23 (3H, each s, 3 × *meso* H), 7.70, 7.64 (1H, m, H-8^2), 7.14 (1H, m, H-8^1), 5.23 (1H, m, H-17), 4.78, 4.62, 4.60, 4.48 (1H, d, d, d, s, H-8^4), 4.40 (2H, m, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 4.19 (1H, m, H-18), 3.61 (2H, m, CH_3CH_2-3), 4.17, 4.95, 4.90, 4.67, 4.62, 4.58, 4.57, 4.16, 4.14, 4.13 (15H, each s, 3 × methyl esters, CH_3-2 and CH_3-12), 2.65, 2.35, 1.95 (1H, 2H, 3H, m, $CH_3OOCCH_2CH_2$-17 and NCH_2CH_2-$CH_2CH_2CH_2CH_3$), 1.68 (6H, m, CH_3CH_2-3 and CH_3-18), 1.58 (2H, m, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 1.54 (3H, splitting s, CH_3-7), 1.43 (4H, m, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 0.95 (3H, t, $J = 7.0$ Hz, $NCH_2CH_2CH_2CH_2CH_2CH_3$), 0.60, 0.58, 0.27, 0.16, 0.14, −0.14, −0.16 (2H, 2 × NH). MS (FAB) *m/z* 803.2 (M^+, 100). HRMS (FAB): Calcd for $C_{46}H_{53}N_5O_8$, 803.3894; Found 803.3895.

Benzobacteriochlorins 20. DMAD (2.0 mL) was added to a solution of 8-Vinylmesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide methyl ester **15** (60 mg) in toluene (20 mL). The mixture was refluxed for 20 h under N_2. The solvent and excess DMAD were removed with rotavapor. The residue was purified with preparative silica TLC using CH_2Cl_2/EtOAc (v/v 30/1) as developing solvent. The intermediate benzobacteriochlorin (20 mg) was obtained, and the unreacted **15** was recovered. The above intermediate compound was dissolved in CH_2Cl_2 (30 mL) and 2 drops of DBU was added to above solution. The resultant solution was stirred at room temperature for 5 min. The solvent was removed and the residue was purified with preparative silica TLC using CH_2Cl_2/EtOAc (v/v 40/1) as developing solvent. The title compound (10 mg) was obtained as a mixture of isomers in 21% converted yield in two steps. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 334 (41 591), 382 (42 244), 438 (68 667), 489 (42 896), 728 (11 417), 805 (34 089). ^{1}H NMR δ 8.99 (1H, s, *meso* H), 8.30 (1H, splitting s, *meso* H), 8.19 (3H, s, 1 × *meso* H and 2 × phenyl H), 7.79 (1H, s, phenyl H), 7.69 (1H, d, $J = 5.9$ Hz, H-8^2), 7.12 (1H, d, $J = 5.6$ Hz, H-8^1), 5.70 [2H, s, CH_2 of bis(trifluoromethyl)benzyl], 5.13 (1H, m, H-17), 4.77 (1H, m, H-8^4), 4.16 (1H, m, H-18), 3.95, 3.60, 3.57, 3.16, 3.11 (each 3H, s, splitting s, s, s, s, CH_3-2, CH_3-12 and 3 × methyl esters), 3.59 (2H, m, CH_3CH_2-3, overlapped with methyls), 2.65, 2.33, 1.89 (1H, 2H, 1H, m, CH_3OOCCH_2-CH_2-17), 1.75−1.60 (6H, m, CH_3CH_2-3 and CH_3-18), 1.56 (3H, s, CH_3-7), 0.88, 0.86, 0.40, 0.38 (total 2H, br s, 2 × NH). ^{19}F NMR δ 13.04 (6F, s, 2 × $-CF_3$). MS (FAB) *m/z* 945.5 (M^+, 100). HRMS (FAB): Calcd for $C_{49}H_{45}F_6N_5O_8$, 945.3172; Found 945.3194.

Intermediate Benzobacteriochlorins 21. DMAD (2.0 mL) was added to a solution of 8-vinylmesochlorin e_6 trimethyl ester **12** (93 mg) in toluene (20 mL). The mixture was refluxed for 5 h under nitrogen. The solvent and excess DMAD were removed with rotavapor. The residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v 20/1) as developing solvent. The title compound (27 mg) was obtained as a mixture of two isomers (6:5) in 34% converted yield. The unreacted **12** (29 mg) was also recovered. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 369 (96 055), 396 (86 594), 454 (4478), 482 (10 906), 511 (12 783), 664 (7439), 731 (49 978). ^{1}H NMR δ 9.06 and 9.00 (each 1H, s, H-10), 8.58 and 8.54 (each 1H, s, H-5), 8.43 and 8.37 (each 1H, s, H-20), 7.16 (1H, dd, $J = 6.4$, 2.8 Hz, H-8^1), 7.10 (1H, dd, $J = 6.7$, 2.2 Hz, H-8^1), 5.15 (2H, dd, AB system, $J = 18.3$ Hz, CH_3OOCCH_2-15), 5.10 (2H, dd, AB system, $J = 18.5$ Hz, CH_3OOCCH_2-15), 4.22, 4.19, 4.04, 3.96, 3.90, 3.88, 3.76, 3.75, 3.65, 3.62, 3.40, 3.37, 3.25, 3.22 (each 3H, s, 4 × ring methyl and 10 × methyl ester), 4.24 (2H, m, 2 × H-18), 4.18 (2H, m, 2 × H-17), 3.92 and 3.52 (each 2H, m, 2 × H-8^2), 3.70 (4H, m, 2 × CH_3CH_2-3), 2.51, 2.18, 1.80 (2H, 4H, 2H, 2 × $CH_3OOCCH_2CH_2$-17), 2.00 and 1.94 (each 3H, s, 2 × CH_3-7), 1.68 (6H, t, $J = 7.5$ Hz, 2 × CH_3CH_2-3), 1.63 (6H, d, $J = 7.2$ Hz, 2 × CH_3-18), −0.62, −0.75, −0.96, −1.13 (each 1H, s, 4 × NH). MS (FAB) *m/z* 780.2 (M^+, 100). HRMS (FAB): Calcd for $C_{43}H_{48}N_4O_{10}$, 780.3370; Found 780.3386.

In Vivo Photosensitizing Activity. C3H/HeJ mice were injected intradermally with 2 × 10^5 RIF cells in 30 μL of Hanks's balanced salt solution without Ca^{2+} and Mg^{2+}, into the flank and allowed to grow until they were 4−5 mm in diameter. The mice were injected (iv) with photosensitizers (5.0 μmol/kg). At 24 h postinjection, the mice were restrained in plastic holders and then treated with laser light from an

argon pumped dye laser in the range of 737−805 nm for a total fluence of 135 J/cm^2 at a fluence rate of 75 mW/cm^2. The mice (5 mice/group) were checked daily, the tumors were measured using two orthogonal measurements L and W (perpendicular to L), and the volumes were calculated using the formula $V = LW^2/2$ and recorded. Mice were considered cured if there was no palpable tumor at 30 days post-PDT treatment.

Peripheral Benzodiazepine Receptor Binding Studies. The experiment was performed as follows. A 50 μL amount of drug solution with decreasing concentrations (3×10^{-4}, 15×10^{-5}, 3×10^{-5}, 15×10^{-6}, 3×10^{-6}, 15×10^{-7}, 3×10^{-7}, 3×10^{-8}, 3×10^{-9}, and 3×10^{-10}) were added into labeled tubes (5 mL disposable glass borosilicate) containing 50 μL of cells (1×10^6) and 50 μL of [^{3}H]-PK11195 (final concentration 46 nM). The resultant samples were incubated at 4 °C for 1 h, and then 3.0 mL of Tris buffer was added to each tube to stop the displacement reactions. The solutions were then filtered by vacuum on GF/C Whatman filters presoaked in Tris buffer 0.5% w/v polyethylenimine (to help prevent nonspecific binding to the filter). The filters were washed with Tris buffer (3×4 mL) and transferred into scintillation vials. A 4 mL amount of scintillation fluid (Universol, ICN) was added to each scintillation vial, shaken to dissolve [^{3}H]-PK11195, and then kept dark to equilibrate for 1 h. The samples were counted in a beta counter (Beckman LS 5801). Control samples replaced cells and/or photosensitizer with Tris buffer. Data were processed with Origin 5.0 (Microcal Software Inc., Northampton, MA).

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