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Synthesis, Spectroscopic and *In-vitro* Photosensitizing Efficacy of Ketobacteriochlorins Derived from Ring-B and Ring-D Reduced Chlorins *via* Pinacol-Pinacolone Rearrangement

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Abstract

In this report we present a regioselective oxidation of a series bacteriochlorins, which on reacting with either ferric chloride (FeCl_3) or 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) yielded the corresponding ring-B or ring-D reduced chlorins. The effect of the number of electron withdrawing groups present at the peripheral position, with or without a fused isocyclic ring (ring-E) did not make any significant difference in regioselective oxidation of the pyrrole rings. However, depending on the nature of substituents, the intermediate bis-dihydroxy bacteriochlorins on subjecting to Pinacol-Pinacolone reaction conditions gave various ketochlorins. The introduction of the keto- group at a particular position in the molecule possibly depends on the stability of the intermediate carbocation species. The newly synthesized bacteriochlorins show strong long-wavelength absorption and produced significant *in vitro* (Colon26 cells) photosensitizing ability. Among the compounds tested, the bacteriochlorins containing a keto-group at position-7 of ring-B with cleaved 5-member isocyclic ring showed the best efficacy.

Introduction

In developing effective agents for Photodynamic Therapy (PDT) the structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) studies have been proven to be extremely useful.¹⁻³ The Roswell Park Cancer Institute (RPCI) group was the first to investigate the SAR and QSAR studies on a series of the alkyl ether analogs of pyropheophorbide-a (chlorophyll-a derivative) and observed a parabolic relationship between overall lipophilicity and PDT efficacy.^{4,5} Among the compounds investigated, 3-(1'-hexyloxyethyl) derivative (HPPH) showed the best PDT efficacy without any significant toxicity and is currently undergoing Phase I/II clinical trials for several indications.⁶⁻⁸ The SAR approach has also been found useful in developing other systems, e. g., phthalocyanines,⁹-tetra (*m*-hydroxyphenyl) chlorins,¹⁰ extended porphyrins (texaphyrins),¹¹ porphyceins,¹² purpurinimides¹³⁻¹⁵ and bacteriopurpurinimides¹⁶ and other bacteriochlorin analogs.^{17,18} The biological studies have indicated that besides overall lipophilicity, the position of the substituents present in the photosensitizer(s) makes a tremendous difference in long-term PDT efficacy.

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Supporting Information Available: ¹H & ¹³C NMR spectra of compounds **10**, **11**, **14-25** and 2D NMR spectra of compounds **11**, **17** and **21**. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

Bacteriochlorins are a class of tetrapyrrolic system in which two pyrrole rings diagonal to each other are reduced.¹⁹ These chromophores exhibit long-wavelength absorption in the range of 720-800 nm depending on the nature of substituents present at the peripheral positions of the molecules. In recent years enormous interest has been generated due to the utility of bacteriochlorins in the bacterial photosynthetic reaction center²⁰ and in the treatment of cancer by photodynamic therapy (PDT). There are several bacteriochlorophyll-a analogs, which are currently under advanced human clinical trials (e.g. Tookad)²¹ or at the advanced preclinical studies (bacteriopurpurinimides) for the treatment of cancer.²² The only naturally occurring bacteriochlorin that is not involved in photosynthesis is the tolyporphyrin isolated by Prinsep et. al. from the blue-green alga *Tolypothrix nodosa*.²³ This compound, which enhances the cytotoxicity of adriamycin or viablastine in SK-VLB cells at doses as low as 1mg/ml, is characterized as a multidrug resistance (MDR) reversing agent. Kishi's group at Harvard University synthesized the tolyporphyrin by the extension of the Eschenmoser sulfide contraction/iminoester cyclization method with long wavelength absorption near 675 nm ($\epsilon = 22,000$)²⁴. However, there are no reports regarding the *in vivo* photosensitizing ability of this novel compound. Lindsey and coworkers²⁵ have also reported facile syntheses of certain bacteriochlorins starting from pyrroles with variable lipophilicity following multistep synthetic methodologies. Some of these compounds exhibit interesting photophysical properties and could be potential candidates for PDT.

One of the simplest methods that has been used extensively for the conversion of porphyrins/chlorins to the corresponding *vic*-dihydroxy chlorins and bacteriochlorins respectively is the osmium tetroxide mediated oxidation.²⁶ These intermediate "diols" on reacting under acidic conditions produce the corresponding keto-chlorins and keto-bacteriochlorins respectively. The position of the keto- group in the resulting chlorins and bacteriochlorins depends on the stability of the intermediate carbocation, which is also influenced by the nature of the substituents (electron withdrawing or electron donating) present in the molecules (Scheme 1).

Chang and Sotiriou²⁷ were the first to show that free-base octaethylchlorin or its metalated analog upon reaction with osmium tetroxide, can be converted into the corresponding ketobacteriochlorins in a reasonable yield. Bonnett et al.²⁸ employed this approach to prepare bacteriochlorin diols by reacting ketoethyl chlorin with osmium tetroxide. Some of these analogs showed considerable PDT efficacy *in vitro*. Pandey et. al. in collaboration with Smith and coworkers extended this approach to the pyropheophorbide-a, purpurin-18 and purpurinimide systems and a series of stable keto-bacteriochlorin analogs were synthesized.²⁹ Most of the resulting bacteriochlorins showed long-wavelength absorption in the range of 730-800 nm and some of them were effective both *in vitro* and *in vivo*.

So far, most of the ketobacteriochlorins [keto- group is present either at position-7 or position-8 (ring-B)] investigated for PDT efficacy are derived from ring-D reduced chlorins³⁰. The main objectives of the work presented herein were (i) to develop an efficient synthetic approach for ring-B reduced chlorins from certain selected bacteriochlorins derived from naturally occurring bacteriochlorophyll-a and then convert them into the corresponding ring-D ketobacteriochlorins (17- keto and 18 keto-), and (ii) to compare the photophysical and photosensitizing abilities of these novel structures.

Results and Discussion

For our present study, two types of bacteriochlorins **6** and **13** containing either a fused five-member isocyclic ring system or a methoxycarbonyl group present at position-13 (ring-C pyrrole) were used as a substrate and these were obtained from bacteriochlorophyll-a by following the literature procedure.³¹ We have recently reported an efficient regioselective

preparation of ring-B and ring-D reduced chlorins from bacteriochlorins.³² We extended this approach for bacteriopheophorbide-a, which on treating with DDQ at room temperature produced mainly D-ring reduced chlorin **5**, whereas on treating **6** with ferric chloride (FeCl₃) produced ring-B reduced chlorin **7** in excellent yields (Scheme 2). Analysis of the NMR data confirmed the structures of both isomers. One of the distinct differences was the resonances of the –CH₂ protons of the five member fused isocyclic ring, which showed an ABX pattern at δ 5.22 ppm in compound **5** due to reduced ring-D, whereas these protons appeared as a singlet at δ 5.44 ppm in compound **7** (ring-D oxidized).

For determining the photophysical and photosensitizing abilities of various ketobacteriochlorin isomers, the keto group was regioselectively introduced either in the ring-B or ring D pyrrole ring of the bacteriochlorin system. For the synthesis of the desired analogs, the ring-D and ring-B reduced chlorins **5** and **7** were individually reacted with OsO₄ and the resulting diols **8** and **10** (both as isomeric mixtures, *cis*-diol up and *cis*-diol down with respect to the diagonal reduced ring) were isolated in 60% yield. Reaction of these diols independently with concentrated sulfuric acid under Pinacol-Pinacolone conditions produced some interesting results. For example diol **8** gave 7-ketobacteriochlorin **9** as a major and **9a** as a minor product and it's exactly matched with the previously reported procedure^{26c} whereas diol **10** under similar reaction conditions gave mainly 18-ketobacteriochlorin **11**. These results suggest that the formation of intermediate carbocation species is certainly directing the position of the keto- group, which is possibly also being influenced by the electron withdrawing acetyl group²⁶ present at position-3 of the 7,8- and 17,18-dihydroxybacteriochlorins (Scheme 3).

For investigating the effect of the variable number of electron withdrawing groups in bacteriochlorin diols under Pinacol-Pinacolone rearrangement conditions, the isocyclic ring in methyl bacteriopheophorbide-a **12** was cleaved on reacting with sodium methoxide and the resulting bacteriochlorin **13** was obtained in excellent yield.³³ Subsequent treatment of **13** with collidine at refluxing temperature gave rhodobacteriochlorin **14** as the minor (15%) and the corresponding ring-B reduced chlorin **15** as the major product (85%), which on reacting with osmium tetroxide/pyridine yielded **17**, 18-bis-dihydroxybacteriochlorin diol **16** as an isomeric mixture (*cis*-hydroxy groups up or down relative to ring-B). Further treatment of **16** with concentrated sulfuric acid gave 18-keto bacteriochlorin **17** as a major product (Scheme 4). As expected, the oxidation of bacteriochlorin **13** with DDQ and FeCl₃ afforded the corresponding ring-D and ring-B reduced chlorins **18** and **22** respectively, which on reacting with osmium tetroxide produced the corresponding diols **19** and **23** in quantitative yields. Interestingly, further reaction of these diols with conc. sulfuric acid gave a mixture of 7-ketobacteriochlorin **20** and 8-ketobacteriochlorin **21** (from **19**) as an isomeric mixture. Separation of these mixtures (**20/21**) by usual chromatographic technique was not successful. Finally, the isomeric mixture was separated by HPLC (column: Luna, eluting solvent: using ethyl acetate and hexane as the eluting solvents) delivered the pure regioisomer 8-ketoisomer **20** and regioisomer 7-ketoisomer **21** were isolated in 19% and 25% respectively. Whereas diol **23** in which the *cis*-hydroxyl groups were present in ring-D yielded only 18-keto bacteriochlorin **17** (in which the β -ketoester functionality present at position-15 was cleaved) as a major product. The elimination of β -ketoester functionality was not observed on treating **19** under similar reaction conditions, and these results were surprising (Scheme 5). The results presented herein confirms our previous findings³⁴, which suggests that the position and presence of the number of electron withdrawing groups present at the peripheral position of chlorin diols makes a significant impact in the stability of the intermediate carbocation during the Pinacol-Pinacolone reaction, which obviously determines the formation of the resulting keto-chlorins under acid catalyzed rearrangement. Therefore in our study to investigate the effect of more than two electron withdrawing groups under acid catalyzed conditions, methyl bacteriopheophorbide-a **12** containing a

methoxycarbonyl group at position-13²-of the fused isocyclic ring was used as a substrate, which on reacting with DDQ and FeCl₃ produced exclusively the ring-D reduced **24** and ring-B reduced chlorins **25** respectively (Scheme 6). Unfortunately, the corresponding bacteriochlorin diols obtained by reacting **24** and **25** with OsO₄/pyridine as such or under acidic conditions at room temperature were not stable and produced complex mixtures, which were not characterized.

The mechanism of the formation of keochlorins under Pinacol-Pinacolone reaction is well established. However the compounds investigated in this study shows some interesting results and mechanism for the formation of these analogs is illustrated in Scheme 7. In brief, in *vic*-dihydroxy analog containing methyl- and ethyl group present at adjacent positions, the migration of methyl group was preferred over the ethyl group and the ethyl group migrated product was isolated in a minor quantity. Interestingly, the *vic*-dihydroxy analogs containing methyl and propionic ester functionality at adjacent positions on treating under similar acidic conditions gave only the methyl migrated product (18-keto-), which of course depend upon the stability of intermediate carbocation species. The previous studies from others^{26c} and our own laboratory³⁴ suggest that in porphyrin system the number of electron withdrawing group present at the peripheral position of the porphyrin skeleton makes a remarkable difference in the stability of intermediate cabocation(s), which dictates the formation of corresponding keto-analogs.

The purity of ketobacteriochlorins **11**, **17**, **20** and **21** was ascertained by HPLC and the structures were assigned by NMR and mass spectrometry analyses. The bacteriochlorins **20** and **21**, which were initially isolated as isomeric mixture, were separated into individual isomers by HPLC using Luna column, eluted with 30% Ethyl Acetate-hexane. The retention time for bacteriochlorins **11**, **17**, **20** and **21** was 12.20, 10.76, 21.13 and 18.47 min respectively (figure 1). A slight shoulder in HPLC chromatogram of **21** could be due to repeated use of the HPLC column for a long time. The purity of the product was also confirmed by using a reverse phase HPLC column (Symmetry C18 column, dimensions 4.6 × 150 mm), eluted with 90%CH₃OH and 10% H₂O and flow rate was adjusted to 1.0 mL/min. For details see “Supporting Material Information”.

A detailed NMR study confirmed the structures of the proposed bacteriochlorins. As can be seen from the results summarized in Figure 2 (only partial NMR Spectra are shown) the *meso*-protons at position 5, 10 and 20 for bacteriochlorins **11**, **17**, **20** and **21** show a significant shift and which could be due to variation in electron density at these positions. Further, in 8-keto bacteriochlorin **20** the 7¹-CH₃ protons appeared as a triplet around δ 0.5 ppm whereas for the other isomer **21**, these protons were observed as a multiplet at δ 0.45-0.51 ppm, which could be due to the presence of epimeric mixture. In compound **20** the 7-CH₃ was observed as a singlet at δ 1.82, while in compound **21**, it exhibited two singlets around δ 1.90/1.92 ppm (epimeric mixture) for 8-CH₃. The 7-CH₂ and 8-CH₂ in isomer **20** and **21** appeared as nice quartets at δ 2.56 and δ 2.62 ppm respectively. These assignments were further confirmed by 2D NMR studies.

In order to confirm the position of dialky groups (methyl/ethyl) or methyl/propionic ester of the major regioisomers, NOESY experiment was performed on isomers **11**, **17** and **21**. In the case of compound **21**, the resonances for the ethyl group at position C-8 were chosen as a starting point for the interpretation of the NOESY results. A peak at δ 2.62 for the CH₂CH₃ protons showed NOE correlation with the adjacent *meso*- proton at δ 8.97 which is C-10; the -CH₃protons of the ethyl group also showed a strong interaction with the methyl proton which is attached to the same carbon (C-8) as well as adjacent *meso*-10-H proton. No correlation was observed between the CH₃ protons and the acetyl protons (C-3), which

further confirmed the proposed structural assignment for **21**. Following a similar approach, the structures of compound **11** and **17** were also established (Figure 3).

Spectroscopic Properties of Keto-Bacteriochlorins

The absorption and fluorescence characteristics of ketobacteriochlorins **9**, **9a**, **11**, **17**, **20** and **21** as epimeric mixtures were measured in dichloromethane. These bacteriochlorins exhibited the long wavelength absorptions at 725, 714, 715, 731, 737 and 732 nm and fluorescence at 736, 727, 732, 735, 765 and 741 respectively. Among all the analogs, the 18-keto-bacteriochlorin **20** exhibited the largest Stokes shift (28 nm), whereas bacteriochlorin **17** lacking the 5-member ring, but instead, containing a methoxycarbonyl- functionality at position-13, showed a much smaller shift of 4 nm. The observed shift was in the order of **20** > **11** > **9a** > **9** > **21** > **17** (Figure 4).

In vitro photosensitizing efficacy

The keto-bacteriochlorins **9**, **9a**, **11**, **17** and **20**, **21** (as an isomeric mixture, and as individual isomers) were evaluated for *in vitro* PDT efficacy in Colon26 tumor cells. The cells were incubated with increasing concentrations of the photosensitizers (3-100 nM) for 24 h and were then exposed to variable light doses (0-2 J/cm²) at an appropriate wavelengths corresponding to the long-wavelength absorption of each compound formulated in 17% BCS in PBS. MTT assay³⁵ was performed after 48 h (for details see the Experimental Section). Among the photosensitizers evaluated, the bacteriochlorins **9**, **9a** containing a keto- group in ring-B, were less effective than bacteriochlorins **11**, **17** containing a keto- group in ring-D. However, the photosensitizer containing a fused 5-member isocyclic ring showed lower activity than **17** bearing a -CO₂Me group at position-13. In contrast bacteriochlorins **20** and **21** (as a 43:57 mixture) containing a keto-group in ring-B showed enhanced activity than **11** and **17**. To investigate the efficacy of the individual isomer, the isomeric mixture of **20** and **21** was duly separated into individual isomers by HPLC (see Figure 1 and Supporting Information) and the *in vitro* photosensitizing efficacy of both the isomers was investigated under similar experimental conditions except the cells were exposed to light at the appropriate long-wavelength absorptions of the photosensitizers (bacteriochlorin **20**, λ_{max} : 737 nm and **21**, λ_{max} : 732 nm). Under similar parameters, isomer **21** showed significantly higher efficacy than the isomer **20** (see inset figure 5C). As mentioned earlier, isomer **21** constitutes a major part of the mixture **20/21** (Scheme 5), which could explain the reason for similar PDT efficacy of the mixture as compared to isomer **21**.

The *in vitro* results depicted in Scheme 6 suggest that besides the position of the keto- group (ring-B vs. ring-D), the nature of the substituents present at the periphery of bacteriochlorin system makes a significant impact in PDT efficacy. However, further study with a series of analogs is required to establish a “true” structure-activity relationship and these studies are currently in progress. Efforts are also underway to investigate a correlation between the cell-uptake, intracellular localization, STAT-3 dimerization³⁶ with *in vitro/in vivo* PDT efficacy and these results will be published in an appropriate journal.

Conclusion

In summary, the work discussed in this manuscript describes an efficient approach for the synthesis of ring-B and ring-D ring reduced chlorins from naturally occurring bacteriochlorophyll-a, which are otherwise difficult to synthesize. Compared to naturally occurring bacteriochlorins, the keto-bacteriochlorins obtained from the respective chlorins showed enhanced stability with significant *in vitro* photosensitizing efficacy. The acetyl group present at position-3 of the chlorin systems provides a unique opportunity to alter the overall lipophilicity of the molecules to investigate the effect of such modifications in *in*

vivo clearance and PDT efficacy. An easy access to these molecules should also generate a great interest in developing new supramolecular structures and synthetic models for understanding the bacterial photosynthetic reaction centers.

Experimental Section

All reactions were carried out in heat gun-dried glassware under an atmosphere of nitrogen with magnetic stirring. Thin-layer chromatography (TLC) was done on precoated silica gel GF PE sheets (layer thickness 0.25 mm) and aluminum oxide NF PE sheets. Column chromatography was performed either over Silica Gel 60 (70-230 mesh) or neutral Alumina. In some cases preparative TLC plates were also used for the purification. Solvents were purified as follows: trace amounts of water and oxygen from THF were removed by refluxing over sodium under an inert atmosphere. Dichloromethane was dried over P₂O₅. Anhydrous DMF, triethylamine, pyridine and other common chromatographic solvents were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on a 400 MHz spectrometer. All chemical shifts are reported in parts per million (δ). ¹H NMR (400 MHz) spectra were recorded at room temperature in CDCl₃ or CD₃OD solutions and referenced to residual CHCl₃ (7.26 ppm) or TMS (0.00 ppm). EI-Mass spectra were carried out on a ion-trap mass spectrometer equipped with a pneumatically assisted electrospray ionization source, operating in positive mode. UV-visible spectra were recorded on FT UV-visible spectrophotometer using dichloromethane/THF as solvent. All photophysical experiments were carried out using spectroscopic grade solvents.

HPLC Method

HPLC analysis of final products was carried out using a Waters Delta 600 System consisting of the 600 Controller, 600 Fluid Handling Unit and 2998 Photodiode Array Detector equipped with a phenomenex Luna column, 5 micron particle size, with dimensions 4.6 $\times$ 250mm. A gradient mobile phase program was used: starting at 30% ethyl acetate /70% hexane linear gradient to 70% ethyl acetate/30% hexane over 80 minutes; the flow rate was 1.0 ml/min.

Methyl 3-acetyl-17,18-dihydroxybacteriopyropheorbide-a 10

Compound **7** (50.0 mg, 0.09 mmol) was taken in a round bottom flask (100 ml) and dissolved in 30 ml dry dichloromethane. To this, added OsO₄ (100.0 mg) and pyridine (1.0 ml) and reaction mixture was stirred vigorously at room temperature for 24 hr. Reaction was monitored by UV-vis and TLC. H₂S gas was then bubbled into the reaction mixture for 5 min. and then excess of H₂S was removed by bubbling N₂ gas for another 30 min. Water was added to reaction mixture and then extracted with dichloromethane (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. Crude thus obtained was chromatographed over silica gel using 0.5-1% CH₃OH/CH₂Cl₂ mixture as eluent to give **10** as a mixture of *cis*-diols (49:51). Yield: 23.0 mg (44 %). ¹H NMR (400 MHz, CDCl₃): δ 9.08 & 9.02 (s, 1H, 5-H), 8.67 & 8.64 (s, 1H, 10-H), 8.30 & 7.96 (s, 1H, 20-H), 5.24 (dd, 1H, 13CHH, *J* = 20 Hz), 4.89 (dd, 1H, 13CHH), 4.30 - 4.25 (m, 1H, 8-H), 4.05 & 3.96 (m, 1H, 7-H), 3.45 & 3.43 (s, 3H, CO₂Me), 3.38 & 3.34 (s, 3H, 12-CH₃), 3.04 & 2.97 (s, 3H, 2-CH₃), 2.89 & 2.24 (s, 3H, COCH₃), 2.96-2.88 (m, 2H, 17¹-CH₂), 2.67 - 2.62 (m, 2H, 17²-CH₂), 2.41 - 2.36 (m, 2H, 8-CH₂-CH₃), 2.15 & 2.12 (s, 3H, 18-CH₃), 1.95 & 1.72 (d, 3H, 7-CH₃, *J* = 7.2 and 7.6 Hz), 1.11 & 1.02 (t, 3H, 8-CH₂CH₃, *J* = 7.2 and 7.6 Hz), -0.09 & -0.03 (brs, 1H, NH), -1.26 & -1.23 (brs, 1H, NH) ¹³C NMR (100 MHz, CDCl₃): δ 199.0, 198.9, 196.1, 196.0, 174.2, 174.1, 170.6, 170.7, 164.8, 164.6, 164.2, 164.0, 154.8, 154.6, 146.9, 146.7, 139.6, 139.1, 137.2, 136.9, 136.6, 136.5, 136.4, 136.1, 132.3, 132.2, 130.3, 129.6, 121.7, 120.9, 109.6, 109.4, 99.2, 99.0, 97.9, 97.8, 94.6, 94.4, 84.3, 83.8,

83.75, 83.74, 55.29, 55.28, 51.8, 51.7, 48.6, 48.5, 47.9, 47.8, 33.24, 33.2, 33.1, 33.0, 30.7, 30.1, 30.0, 28.9, 28.8, 23.1, 22.9, 20.9, 20.7, 11.0, 10.8, 10.7, 10.4. EIMS (m/z): 621 (M^+ +Na). HRMS: Calcd. For $C_{34}H_{39}N_4O_6[MH]^+$: 599.2870. Found: 599.2880.

Methyl 3-acetyl-18-keto-bacteriopheophorbide-a 11

Compound **10** (15.0 mg, 0.02 mmol) was taken in a round bottom flask (100 ml) and dissolved in 15 ml conc. H_2SO_4 . The reaction mixture was stirred vigorously at room temperature for 30 min and the poured into ice-water. Reaction mixture was then extracted with dichloromethane (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. Crude thus obtained was chromatographed over silica gel using 1-3% CH_3OH/CH_2Cl_2 mixture as eluent to get **11**. Yield: 6.0 mg (42.8 %). UV-vis (THF, λ_{max} (nm), ϵ): 715 (9.27×10^4), 650 (2.19×10^4), 518 (2.19×10^4), 432 (1.48×10^5) and 385 (1.39×10^5). 1H NMR (400 MHz, $CDCl_3$): δ 9.06 (s, 1H, 5-H), 8.55 (s, 1H, 10-H), 8.51 (s, 1H, 20-H), 5.32 (s, 2H, 13- CH_2), 4.38-4.33 (m, 1H, 7-H), 4.07-4.11 (m, 1H, 8-H), 3.56 (s, 3H, CO_2Me), 3.46 (s, 3H, 12- CH_3), 3.38 (s, 3H, 2- CH_3), 3.18 (s, 3H, $COCH_3$), 2.86-2.80 (m, 2H, 17^1-CH_2), 2.41 - 2.35 (m, 1H, 8- $CHH-CH_3$), 2.17 - 2.06 (m, 3H, 17^2-CH_2 & 1H of 8- $CHH-CH_3$), 1.89/1.88 (s, 3H, 17- CH_3), 1.83 (d, 3H, 7- CH_3 $J = 6.4$ Hz), 1.10-1.15 (distorted triplet, 3H, 8- CH_2CH_3), 0.2 (brs, 1H, NH), -0.88 (brs, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.7, 199.0, 196.5, 173.1, 165.9, 165.6, 144.9, 141.9, 138.7, 138.2, 138.1, 134.0, 133.8, 128.0, 122.2, 115.0, 112.9, 99.3, 97.6, 94.0, 55.0, 54.7, 51.4, 49.2, 47.9, 33.3, 32.4, 30.1, 28.9, 22.4, 22.4, 13.6, 11.5, 10.7; EIMS (m/z): 603 (M^+ +Na). HRMS: Calcd. For $C_{34}H_{37}N_4O_5[MH]^+$: 581.2764 Found: 581.2770.

Methyl bacteriopheophorbide-a 12

Rhodobacter sphaeroides (containing bacteriochlorophyll *a* (**6**)) biomass (~500 gram) was suspended in 1-propanol (2 L) and stirred at room temperature in dark with constant nitrogen bubbling for 12 hours. The blue-green extract was filtered and aqueous 0.5 N HCl (150 to 200 ml) was added to the filtrate. After stirring for 25 minutes, the solution began to turn reddish. The reaction mixture was then diluted with aqueous 5% NaCl (1.5 L) and extracted with dichloromethane. The combined extracts were washed with water, dried and rotavaporated. The residue was precipitated from hexanes to give crude bacteriopheophytin-*a* **6** (2 g) with purity sufficient to proceed to the next step. Compound **6** was dissolved in aqueous 80% TFA (300 ml) and stirred in the dark under N_2 at $0^\circ C$ for 2 hours. The solution was then diluted with ice/water (600 ml) and extracted with dichloromethane. The combined organic extracts were washed with water, treated with diazomethane and evaporated to dryness. The crude residue was precipitated from hexanes to obtain the title compound (1.20 g), m. p. 222-224 $^\circ C$. UV-vis (ethyl ether, λ_{max} nm (ϵ)): 358 (11.8×10^4), 385 (6.76×10^4), 525 (2.89×10^4), 680 (1.22×10^4), 749 (6.75×10^4); (in CH_2Cl_2): 362 (10.8×10^4), 389 (5.81×10^4), 530 (2.84×10^4), 683 (1.11×10^4), 754 (6.27×10^4). 1H NMR δ (in $CDCl_3$): 8.98 (s, 1H, 5-H), 8.49 (s, 1H, 10-H), 8.41 (s, 1H, 20-H), 6.08 (s, 1H, 13^2-H), 4.27 (m, 2H, 1H for 7-H, 1H for 8-H), 4.02 (m, 2H, 1H for 17-H, 1H for 18-H), 3.85 (s, 3H, 12- CH_3), 3.59 (s, 3H, 2- CH_3), 3.49 (s, 3H, $13^2-COOCH_3$), 3.45 (s, 3H, 17- CH_2CH_2 $COOCH_3$), 3.16 (s, 3H, 3- $COCH_3$), 2.52 (m, 2H, 17- CH_2CH_2 $COOCH_3$), 2.34 (m, 2H, 17- CH_2CH_2 $COOCH_3$), 2.25 (m, 2H, 8- CH_2CH_3), 1.80 (d, $J = 7.4$ Hz, 3H, 7- CH_3), 1.73 (d, $J = 7.9$ Hz, 3H, 18- CH_3), 1.12 (t, $J = 7.2$ Hz, 3H, 8- CH_2CH_3), 0.47 (s, 1H, NH), -0.95 (s, 1H, NH). EIMS (m/z): 626 ($M+1$). HRMS: $C_{36}H_{41}N_4O_6 [MH]^+$: Calcd 625.3026. Found. 625.3043.

3-Acetyl-bacteriochlorin 15-glyoxilic acid trimethyl ester 13

Methyl bacteriopheophorbide-a **12** (100 mg, 016 mmol) was taken in a round bottom flask (100 ml) and dry THF (30 ml) added. 0.3 ml of NaOMe (25% in CH_3OH) was dissolved in 10 ml of dry THF and added slowly via syringe to the reaction mixture under vigorous stirring. The reaction mixture was stirred at room temperature for 4 hr and was quenched

with 5% acetic acid-H₂O and extracted with dichloromethane (100 ml). Organic layer separated, washed with brine, dried over sodium sulfate and concentrated to dryness. Trace of acetic acid was removed under high vacuum. Crude product was re-dissolved in dichloromethane and treated with diazomethane. Reaction mixture was stirred for 10 min and then excess of diazomethane was removed by bubbling N₂ gas. Reaction mixture concentrated and chromatographed over silica gel using 1-3% CH₃OH/dichloromethane gradient as eluent to obtained compound as major product. Slow moving brown-red band on silica. Yield: 40.0 mg (37.2%), m.p. > 260°C (decomp.); UV-vis $\lambda_{\max}$ (in CH₂Cl₂): 782 nm (ϵ 5.19×10^4), 748 (10.4×10^4), 543 (3.12×10^4), 410 (5.27×10^4) and 363 (7.61×10^4); ¹HNMR (400 MHz, CDCl₃): δ 9.14 (s, 1H, meso-H), 8.68 (s, 1H, meso-H), 8.54 (s, 1H, meso-H), 4.48 (m, 1H, 17-H), 4.26 (m, 1H, 8-H), 4.22 (m, 1H, 18-H), 4.10 (s, 3H, CO₂Me), 4.07 (m, 1H, 7-H), 3.91 (s, 3H, CO₂Me), 3.53 (s, 3H, CO₂Me), 3.51 (s, 3H, 12-CH₃), 3.44 (s, 3H, 2-CH₃), 3.15 (s, 3H, COCH₃), 2.31 (m, 2H, 17²-CH₂), 2.06-2.00 (m, 3H, 8-CH₂CH₃ & 17¹-CH₂), 1.83 (d, 3H, 7-CH₃, $J = 7.2$ Hz), 1.76 (d, 3H, 18-CH₃, $J = 7.6$ Hz), 1.72 (m, 1H, 17¹-CH₂), 1.06 (t, 3H, 8-CH₂CH₃, $J = 7.6$ Hz), -0.45 (brs, 1H, NH), -0.53 (brs, 1H, NH). EIMS (m/z): 671.3 (M+H). HRMS: Calcd. For C₃₇H₄₂N₄O₈: 670.3002. Found: 670.3030.

Rhodobacteriochlorin 14

The second band from the column. Yield: 9.0 mg (10.2%). UV-vis (CH₂Cl₂, $\lambda_{\max}$, nm, (ϵ): 355.0 (1.05×10^5), 521.0 (2.22×10^4), 760 (8.43×10^4) ¹HNMR (400 MHz, CDCl₃): δ 9.58 (s, 1H, meso-H), 9.32 (s, 1H, meso-H), 8.72 (s, 1H, meso-H), 8.65 (s, 1H, meso-H), 4.43-4.33 (m, 3H, 8-H, 17-H, 18-H), 4.31 (s, 3H, CO₂Me), 4.19-4.16 (m, 1H, 7-H), 3.63 (s, 3H, CO₂Me), 3.62 (s, 3H, ring-CH₃), 3.58 (s, 3H, ring-CH₃), 3.20 (s, 3H, COCH₃), 2.67-2.56 (m, 2H, 17²-CH₂), 2.42-2.35 (m, 3H, 8-CH₂CH₃ & 17¹-CH₂), 2.13-2.07 (m, 1H, 17¹-CH₂), 1.83 (d, 3H, 7-CH₃, $J = 4.4$ Hz), 1.81 (d, 3H, 18-CH₃, $J = 4.4$ Hz), 1.11 (t, 3H, 8-CH₂CH₃, $J = 7.6$ Hz), -1.39 (brs, 1H, NH), -1.44 (brs, 1H, NH). EIMS (m/z): 584.5 (M⁺). ¹³C NMR (100 MHz, CDCl₃): δ 198.8, 173.8, 168.5, 166.9, 166.0, 164.8, 164.6, 135.6, 135.0, 134.6, 133.9, 133.7, 132.2, 129.7, 119.7, 98.6, 98.5, 97.2, 96.3, 56.8, 54.8, 51.5, 47.6, 47.5, 33.2, 32.1, 30.9, 30.2, 29.6, 23.6, 23.5, 13.6, 13.2, 10.8. HRMS: Calcd. For C₃₄H₄₁N₄O₅[MH]⁺: 585.3077. Found: 585.3059.

Ring-B reduced chlorin 15

3-Acetyl-bacteriochlorin 15-glyoxilic acid trimethyl ester **13** (100.0 mg, 0.15 mmol) was refluxed in collidine (15 ml) for 30 min. Progress of the reaction was monitored by UV-vis and TLC. After completion, reaction mixture was concentrated to dryness using high vacuum and purified on alumina (G-III) column using dichloromethane-hexane mixture as eluent. Yield: 50.0 mg (57.6%). UV-vis (CH₂Cl₂, $\lambda_{\max}$, nm, (ϵ): 410.0 (8.16×10^4), 509.0 (7.50×10^3), 683.1 (4.16×10^4) ¹HNMR (400 MHz, CDCl₃): δ 10.50 (s, 1H, meso-H), 9.71 (s, 1H, meso-H), 9.60 (s, 1H, meso-H), 8.95 (s, 1H, meso-H), 4.60 (m, 1H, 8-H), 4.40 (s, 3H, CO₂Me), 4.36 (m, 1H, 7-H), 4.12 (t, 2H, 17¹-CH₂, $J = 7.6$ Hz), 3.77 (s, 3H, ring-CH₃), 3.75 (s, 3H, ring-CH₃), 3.70 (s, 3H, CO₂Me), 3.35 (s, 3H, ring-CH₃), 3.26 (s, 3H, COCH₃), 3.18 (t, 2H, 17²-CH₂, $J = 7.6$ Hz), 2.51-2.46 (m, 1H, 8-CH₂CH₃), 2.20-2.15 (m, 1H, 8-CH₂CH₃), 1.92 (d, 3H, 7-CH₃, $J = 7.6$ Hz), 1.13 (t, 3H, 8-CH₂CH₃, $J = 7.6$ Hz), -1.79 (brs, 2H, NH). ¹³C NMR (100 MHz, CDCl₃): δ 198.8, 173.5, 171.5, 168.9, 166.5, 152.7, 151.9, 140.8, 138.7, 138.1, 137.9, 137.3, 137.2, 133.5, 132.1, 130.1, 124.3, 101.9, 100.9, 96.7, 94.9, 57.4, 51.6, 47.8, 37.0, 33.3, 30.2, 29.6, 23.8, 21.9, 14., 13.2, 11.5, 10.8. EIMS (m/z): 583.8 (M⁺+1). HRMS: Calcd. For C₃₄H₃₉N₄O₅[MH]⁺: 583.2920. Found: 583.2938.

3-Acetyl-17,18-bis hydroxyl-ring-B reduced chlorin 16

Acetyl-chlorin-dimethyl ester **15** (50.0 mg, 0.08 mmol) was taken in a round bottom flask (100 ml) and dissolved in 30 ml dry dichloromethane. To this were added OsO₄ (100.0 mg)

and pyridine (1.0 ml) and reaction mixture was stirred vigorously at room temperature for 24 hr. Reaction was monitored UV-vis and TLC. H₂S gas was then bubbled into the reaction mixture for 5 min. and then excess of H₂S was removed by bubbling N₂ gas for 30 min. Added water to reaction mixture and extracted with dichloromethane (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. The crude reaction product thus obtained was chromatographed over silica gel using 0.5-1% CH₃OH/CH₂Cl₂ mixture as eluent to give product as a mixture of *cis*-diols (44:56). Yield: 30.0 mg (57%). UV-vis $\lambda_{\max}$ (in CH₂Cl₂) (ϵ): 358.1 nm (7.88×10^4), 388.1 nm (7.35×10^4), 524 nm (2.49×10^4), 751.9 nm (7.88×10^4). ¹H NMR (400 MHz, CDCl₃): δ 9.72 & 9.69 (s, 1H, meso-H), 9.34 & 9.33 (s, 1H, meso-H), 8.83 & 8.79 (s, 1H, meso-H), 8.71 & 8.64 (s, 1H, meso-H), 4.43-4.36 (m, 1H, 8-H), 4.27 & 4.20 (m, 1H, 7-H), 4.12 & 4.10 (s, 3H, CO₂Me), 3.67 & 3.61 (s, 3H, CO₂Me), 3.49 & 3.36 (s, 3H, ring-CH₃), 3.31 (s, 3H, ring-CH₃), 2.90 & 2.87 (s, 3H, COCH₃), 2.85 (m, 2H, 17¹-CH₂), 2.76 & 2.66 (m, 2H, 17²-CH₂), 2.41 & 2.31 (m, 1H, 8-CHH-CH₃), 2.11 & 2.00 (m, 1H, 8-CHH-CH₃), 2.06 & 1.87 (s, 3H, 18-CH₃), 1.91 & 1.78 (d, 3H, 7-CH₃, $J = 7.6$ Hz), 1.15 & 1.08 (t, 3H, 8-CH₂CH₃, $J = 7.6$ Hz), -1.30 & -1.37 (brs, 1H, NH), -1.42 & -1.44 (brs, 1H, NH). ¹³C NMR (100 MHz, CDCl₃): δ 198.7, 198.6, 177.4, 177.3, 175.0, 170, 169.2, 169.1, 166.9, 166.3, 165.0, 163.9, 158.5, 158.4, 154.4, 154.2, 153.0, 143.0, 137.1, 137.0, 135.7, 135.6, 135.5, 135.4, 134.9, 134.8, 133.3, 133.4, 120.0, 119.9, 99.4, 99.2, 98.5, 98.3, 95.0, 96.1, 94.8, 94.2, 86.0, 86.4, 84.1, 84.0, 56.9, 57.1, 56.6, 56.5, 52.0, 51.9, 48.1, 47.8, 33.2, 33.1, 30.3, 30.2, 29.9, 30.0, 29.3, 29.2, 25.0, 25.1, 23.5, 23.3, 13.67, 13.64, 13.36, 13.14, 10.69, 10.64; EIMS (m/z): 617 ($M^+ + 1$). HRMS: Calcd. For C₃₄H₄₀N₄O₇[MH]⁺ : 617.2975. Found: 617.2980.

3-Acetyl-18-ketobacteriochlorin 17

Bacteriochlorin diol **16** (25.0 mg, 0.04 mmol) was taken in a round bottom flask (100 ml) and dissolved in 15 ml conc. H₂SO₄. The reaction mixture was stirred vigorously at room temperature for 30 min and then poured into ice-water. Reaction mixture was then extracted with dichloromethane (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. Crude thus obtained was chromatographed over silica gel using 1-3 % CH₃OH/CH₂Cl₂ mixture as eluent to get the product; Yield: 15.0 mg (61.9%). UV-vis (CH₂Cl₂, $\lambda_{\max}$, nm, (ϵ): 403.1 (7.68×10^4), 425.0 (9.10×10^4), 507.0 (1.17×10^4), 539.0 (2.72×10^3), 725.00 (8.70×10^4). ¹H NMR (400 MHz, CDCl₃): δ 10.30 (s, 1H, 5-H), 9.44 (s, 1H, 10-H), 8.88 (s, 1H, 20-H), 8.89 (s, 1H, 15-H), 4.48-4.51 (m, 1H, 7-H), 4.37 (s, 3H, 17-CO₂Me), 4.24-4.28 (m, 1H, 8-H), 3.69 (s, 3H, 13-CO₂Me), 3.68 (s, 3H, 2-CH₃), 3.32 (s, 3H, 12-CH₃), 3.24 (s, 3H, COCH₃), 2.99-2.95 (m, 2H, 17¹-CH₂), 2.49 - 2.39 (m, 2H, 8-CHH-CH₃ & 1H of 17²-CH₂), 2.20 - 2.10 (m, 2H, 8-CHH-CH₃ & 17²-CH₂), 1.95/1.97 (singlets, 3H, 17-CH₃), 1.86 (d, 3H, 7-CH₃, $J = 7.2$ Hz), 1.09-1.15 (m, 3H, 8-CH₂CH₃), -1.50 (brs, 1H, NH), -1.6 (brs, 1H, NH). ¹³C NMR (100 MHz, CDCl₃): δ 208.6, 198.7, 173.1, 172.0, 166.5, 166.3, 162.8, 145.2, 136.8, 136.4, 135.1, 132.2, 131.5, 127.9, 123.4, 114.6, 98.6, 97.6, 94.6, 56.7, 53.4, 53.0, 52.2, 51.3, 48.3, 33.3, 30.4, 29.7, 28.8, 23.4, 23.0, 13.9, 12.9, 10.8; EIMS (m/z): 621.3 ($M + Na$). HRMS: C₃₄H₃₉N₄O₆[MH]⁺, Calcd. 599.2870 Obsd. 599.2882.

3-Aetyl-chlorin-15-glyoxilic acid trimethyl ester 18

3-Acetyl-bacteriochlorin 15-glyoxilic acid trimethyl ester **13** (45 mg, 0.06 mmol) was dissolved in dichloromethane (15 ml). To this mixture was added slowly a CH₂Cl₂ solution of DDQ (30 mg, 0.13 mmol). The resulting mixture was stirred at room temperature for 10 min and washed with water three times. The organic layer was separated and dried over anhydrous Na₂SO₄ and solvent was removed under vacuum. The residue obtained was purified with preparative plates using 2% acetone/dichloromethane. Yield: 35.0 mg (78.6 %). UV-vis (CH₂Cl₂, $\lambda_{\max}$, nm, (ϵ): 410.9 (8.48×10^4), 504.9 (7.24×10^3), 546 (8.77×10^3), 692 (2.79×10^4). ¹H NMR δ (in CDCl₃): 9.94 (s, 1H, 5H), 9.68 (s, 1H, 10H), 8.70 (s, 1H, 20H),

4.66 (dd, 1H, 18 H, $J = 6.8$ Hz), 4.35 (m, 1H, 17H, $J = 7.2$ Hz), 4.15 (s, 3H, CO₂Me), 3.90 (s, 3H, CO₂Me), 3.72 (m, 3H, 8 CH₂ + 17¹-CH₂), 3.66 (s, 3H, CO₂Me), 3.53 (s, 3H, 12 CH₃), 3.73 (s, 3H, 2 CH₃), 3.25 (s, 3H, 7-CH₃), 3.20 (s, 3H, COCH₃), 2.05- 2.17 (m, 3H, 17²-CH₂ + 17¹-CH₂), 1.68 (t, 3H, 8CH₃, $J = 7.6$ Hz), 1.81 (d, 3H, 18 CH₃, $J = 8$ Hz), 1.68 (t, 3H, 8CH₃, $J = 7.6$ Hz). ¹³C NMR (100 MHz, CDCl₃): δ 198.5, 186.4, 173.2, 171.4, 167.4, 166.4, 163.5, 155.4, 150.1, 145.3, 140.6, 139.9, 137.4, 136.7, 135.6, 135.1, 135.0, 131.4, 121.1, 106.6, 105.4, 104.3, 94.7, 53.3, 52.8, 52.1, 51.5, 49.2, 33.2, 31.4, 30.9, 23.2, 19.4, 17.4, 13.4, 13.0, 11.1. EIMS (m/z): 691.1 (M⁺ + Na). HRMS: Calcd. For C₃₇H₄₁N₄O₈[MH]⁺: 669.2924. Found: 669.2916.

3-Acetyl-7,8-dihydroxybacteriochlorin-15-glyoxilic acid trimethyl ester 19

Compound **18** (30.0 mg, 0.04 mmol) was taken in a round bottom flask (100 ml) and dissolved in 30 ml dry dichloromethane (DCM). To this were added OsO₄ (100 mg) and pyridine (1.0 ml) and reaction mixture was stirred vigorously at room temperature for 24 hr. Reaction was monitored UV-vis and TLC. H₂S gas was then bubbled into the reaction mixture for 5 min and then excess of H₂S was removed by bubbling N₂ gas for 30 min. Added water to reaction mixture and extracted with DCM (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. Crude thus obtained was purified with silica gel preparative plates using 5% CH₃OH/CH₂Cl₂ to give **19** as a mixture of *cis*-diols (38:62). Yield: 27.0 mg (64%). UV-vis (CH₂Cl₂, $\lambda_{\max}$, nm, ϵ): 761 (7.1×10^4), 529 (3.47×10^4), 386 (9.9×10^4), 358 (1.3×10^5). ¹H NMR δ (in CDCl₃): 9.31 & 9.28 (s, 1H, 5H), 8.95 & 8.93 (s, 1H, 10H), 8.55 & 8.50 (s, 1H, 20H), 4.37-4.39 (m, 1H, 18 H), 4.25-4.26 (m, 1H, 17H), 4.09 & 4.07 (s, 3H, CO₂Me), 3.93 & 3.90 (s, 3H, CO₂Me), 3.72-3.60 (m, 3H, 8 CH₂ + 17¹-CH₂), 3.56 & 3.53 (s, 3H, CO₂Me), 3.45 & 3.43 (s, 3H, 12 CH₃), 3.38 & 3.34 (s, 3H, 2 CH₃), 2.97 & 2.84 (s, 3H, 7 CH₃), 2.26- 2.41 (m, 3H, 17²-CH₂ + 17¹-CH₂), 2.10 & 2.22 (s, 3H, COCH₃), 1.67 & 1.80 (d, 3H, 18-CH₃, $J = 7.6$ Hz), 0.68 & 0.86 (t, 3H, 8-CH₃, $J = 7.2$ and 7.6 Hz), -0.41 & -0.44 (brs, 1H, NH), -0.49 & -0.53 (brs, 1H, NH); ¹³C NMR (100 MHz, CDCl₃): δ 198.2, 198.1, 186.4, 186.3, 173.3, 173.1, 169.7, 168.8, 166.6, 166.5, 166.1, 166.0, 165.8, 165.7, 164.8, 164.3, 163.2, 163.3, 161.5, 160.4, 136.0, 136.1, 135.9, 135.7, 134.3, 133.2, 132.7, 132.4, 132.0, 131.9, 131.5, 131.2, 120.7, 120.1, 108.8, 108.7, 100.3, 100.2, 98.6, 97.1, 96.5, 86.3, 85.8, 84.9, 82.7, 82.4, 53.3, 52.8, 52.1, 52.0, 51.9, 51.7, 51.6, 51.5, 49.1, 49.0, 32.9, 32.8, 31.3, 31.2, 31.0, 30.9, 22.7, 22.6, 20.7, 20.0, 13.8, 13.7, 13.3, 13.2, 12.8, 12.7, 8.3, 8.2; EIMS (m/z): 703 (M+H). HRMS: Calcd. For C₃₇H₄₃N₄O₁₀[MH]⁺: 703.2979. Found: 703.3000.

3-Acetyl-8-keto- and 3-Acetyl-7-ketobacteriochlorin-15-glyoxilic acid trimethyl ester 20 and 21

Compound **19** (20.0 mg, 0.04 mmol) was taken in a round bottom flask (100 ml) and dissolved in 15 ml conc. H₂SO₄. The reaction mixture was stirred vigorously at room temperature for 30 min and the poured into ice-water. Reaction mixture was then extracted with dichloromethane (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. Crude thus obtained was chromatographed using preparative plates using 50% Ethyl acetate/hexane to give an isomeric mixture of **20** & **21** (43:57). This isomeric mixture was then separated by HPLC using the conditions described above in the experimental sections to yield pure isomer of **20** and **21**.

3-Acetyl-8-ketobacteriochlorin-15-glyoxilic acid trimethyl ester (20)

Yield: 3.8mg (19%). UV-vis (CH₂Cl₂, $\lambda_{\max}$, nm, ϵ): 392.1 (1.24×10^5), 508.0 (1.0×10^4), 546.0 (2.0×10^3), 737.0 (5.1×10^4). ¹H NMR (400 MHz, CDCl₃): δ 9.45 (s, 1H, 5-H), 9.10 (s, 1H, 10-H), 8.51 (s, 1H, 20-H), 4.43-4.45 (m, 1H, 18-H), 4.21- 4.27 (m, 1H, 17-H), 4.11 (s, 3H, CO₂Me), 3.91 (s, 3H, CO₂Me), 3.56, 3.53 and 3.51 (each s, 3H, 12-CH₃, 2-CH₃ & CO₂Me), 3.18 (s, 3H, COCH₃), 2.56 (q, 2H, 7-CH₂, $J = 8$ Hz), 2.36-2.37 (m, 1H, 17¹-CH₂),

2.04- 2.09 (m, 3H, 2H of 17²-CH₂ and 1H of 17¹-CH₂), 1.82 (s, 3H, 7-CH₃), 1.74 (d, 3H, 18-CH₃, *J* = 7.6 Hz), 0.50 (t, 3H, 7-CH₂CH₃, *J* = 8 Hz), 0.19 (brs, 1H, NH), 0.13 (brs, 1H, NH). ¹³C NMR (100 MHz, CDCl₃): δ 209.2, 198.0, 185.8, 174.1, 173.2, 168.4, 166.4, 166.1, 163.25, 143.1, 140.39, 137.8, 137.5, 137.4, 134.7, 133.8, 129.3, 127.8, 101.3, 98.4, 96.03, 55.1, 53.3, 52.0, 51.6, 49.9, 33.1, 31.5, 31.4, 31.2, 31.04, 30.9, 29.6, 22.6, 22.5, 13.4, 12.9, 12.8, 8.8; EIMS (*m/z*): 707.3 (M⁺ + Na). HRMS: Calcd. For C₃₇H₄₁N₄O₉[MH]⁺: Calcd: 685.2874 Found: 685.2890.

3-Acetyl-7-ketobacteriochlorin-15-glyoxilic acid trimethyl ester (21)

Yield: 4.5mg (25%). UV-vis (CH₂Cl₂, λ_{max}, nm, (ε): 385 (1.19 × 10⁵), 428.0 (1.29 × 10⁵), 511.0 (1.96 × 10⁴), 544.0 (1.08 × 10⁴), 732.0 (9.1 × 10⁴). ¹H NMR (400 MHz, CDCl₃): δ 9.83 (s, 1H, 5-H), 8.97 (s, 1H, 10-H), 8.81 (s, 1H, 20-H), 4.49-4.52 (m, 1H, 18-H), 4.35- 4.37 (m, 1H, 17-H), 4.15 (s, 3H, CO₂Me), 3.97/3.96 (s, 3H, CO₂Me), 3.58 & 3.54 (s, 9H, 12 CH₃, 2-CH₃ & CO₂Me), 3.29 (s, 3H, COCH₃), 2.62 (q, 2H, 8-CH₂CH₃, *J* = 7.8 Hz), 2.36-2.37 (m, 1H, 17¹ -CH₂), 2.05- 2.09 (m, 3H, 2H of 17²-CH₂ and 1H of 17¹-CH₂), 1.90/1.92 (s, 3H, 8-CH₃), 1.78-1.80(m, 3H, 18-CH₃), 0.45-0.51 (m, 3H, 8-CH₂CH₃), -1.07 (brs, 1H, NH), -1.01 (brs, 1H, NH). ¹³C NMR (100 MHz, CDCl₃): δ 208.7, 198.1, 198.1, 186.4, 173.0, 169.2, 166.7, 166.3, 163.0, 162.95, 162.93, 147.7, 139.4, 136.7, 136.6, 135.6, 135.2, 134.6, 134.2, 133.3, 133.2, 98.9, 98.8, 97.3, 54.6, 53.2, 52.4, 51.6, 48.8, 33.3, 31.8, 31.7, 31.3, 30.5, 29.7, 23.2, 23.0, 22.9, 13.1, 13.0, 8.8, 8.7; EIMS (*m/z*): 707.3 (M⁺ + Na). HRMS: Calcd. For C₃₇H₄₁N₄O₉[MH]⁺: Calcd: 685.2874 Found: 685.2852.

3-Acetyl-15-glyoxilic acid-ring-B reduced chlorin trimethyl ester 22

Acetyl-bacteriochlorin 15-glyoxilic acid trimethyl ester **13** (45 mg, 0.06 mmol) was dissolved in dichloromethane (20 ml). To this mixture was added slowly a nitromethane solution of FeCl₃·6H₂O (72 mg, 0.267 mmol). The resulting mixture was stirred at room temperature for 30 min, quenched by addition of 5ml methanol, and washed with water three times. The organic layer was separated and dried over anhydrous Na₂SO₄ and solvent was removed under vacuum. The residue obtained was purified with preparative plates using 2% acetone/dichloromethane Yield: 39.0 mg (87.2%). UV-vis (CH₂Cl₂, λ_{max}, nm, (ε): 410.9 (8.09 × 10⁴), 512.1 (1.00 × 10⁴), 679 (3.28 × 10⁴). ¹H NMR δ (in CDCl₃): 9.85 (s, 1H, 5H), 9.56 (s, 1H, 10H), 8.90 (s, 1H, 20H), 4.53 (q, 1H, 7-H, *J* = 24 Hz), 4.35 (m, 1H, 8H), 4.15 (s, 3H, CO₂Me), 3.90 (s, 3H, CO₂Me), 3.80 (s, 3H, CO₂Me), 3.63 (s, 3H, 12 CH₃), 3.52 (s, 3H, 2 CH₃), 3.32 (s, 3H, 18 CH₃), 3.26 (s, 3H, COCH₃), 2.58-2.64 (m, 2H, 17¹-CH₂), 2.43-2.45 (m, 1H, 8-CHH), 2.10-2.14 (m, 1H, 8-CHH), 1.89 (d, 3H, 7CH₃, *J* = 6.8 Hz), 1.08 (t, 3H, 8-CH₂CH₃, *J* = 7.6 Hz), -1.2 (brs, 1H, NH), -1.60 (brs, 1H, NH). ¹³C NMR (100 MHz, CDCl₃): δ 198.4, 189.3, 173.0, 172.0, 169.9, 167.0, 161.8, 149.6, 149.4, 141.8, 138.5, 138.2, 137.8, 137.7, 137.4, 131.0, 130.8, 129.9, 127.5, 113.0, 102.8, 97.8, 96.0, 58.0, 54.0, 53.5, 51.6, 47.1, 35.0, 33.2, 30.0, 23.6, 23.4, 14.0, 12.6, 11.8, 10.8. EIMS (*m/z*): 691.1 (M⁺ + Na). HRMS: Calcd. For C₃₇H₄₁N₄O₈[MH]⁺: 669.2924. Found: 669.2912.

3-Acetyl-17,18-dihydroxy-15-glyoxilic acid-bacteriochlorin trimethyl ester 23

Compound **22** (40.0 mg, 0.06 mmol) was taken in a round bottom flask (100 ml) and dissolved in 30 ml dry DCM. To this were added OsO₄ (75.0 mg) and pyridine (1.0 ml) and reaction mixture was stirred vigorously at RT for 24 hr. Reaction was monitored by UV-vis and TLC. H₂S gas was then bubbled into the reaction mixture for 5 min. and then excess of H₂S was removed by bubbling N₂ gas for another 30 min. Water was added to reaction mixture and extracted with dichloromethane (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. Crude thus obtained was purified with silica gel preparative plates using 5% MeOH/CH₂Cl₂. Yield: 23 mg (55%). ¹H NMR δ (in CDCl₃): 9.45 & 9.43 (s, 1H, 5H), 8.88 & 8.86 (s, 1H, 10H), 8.75 & 8.73 (s, 1H, 20H), 4.38-4.40 (m, 1H, 8 H), 4.28-4.29 (m, 1H, 7H), 4.16 & 4.15 (s, 3H, CO₂Me), 3.63 & 3.67 (s,

3H, CO₂Me), 3.51 & 3.50 (s, 3H, CO₂Me), 3.43 & 3.42 (s, 3H, 12 CH₃), 3.14 & 3.13 (s, 3H, 2 CH₃), 3.10 & 3.09 (s, 3H, 18 CH₃), 2.52 (s, 3H, 3COCH₃), 2.31- 2.24 (m, 2H, 17-CH₂), 2.19- 2.24 (m, 2H, 8-CH₂), 2.04- 2.08 (m, 2H, 17-CH₂), 1.73 & 1.89 (d, 3H, 7 CH₃, *J* = 7.2 and 7.6 Hz), 1.08 (t, 3H, 8CH₃, *J* = 5.6 Hz), -0.354 & -0.415 (brs, 1H, NH), -0.52 & -0.60 (brs, 1H, NH), ¹³C NMR (100 MHz, CDCl₃): δ 198.3, 198.4, 175.2 (2C ?), 174.4, 174.3, 171.0, 170.9, 170.1, 170.0, 167.2, 167.1, 166.2, 166.1, 160.2, 159.8, 159.5, 159.4, 158.2, 157.8, 153.0, 143.0, 137.4, 137.2, 135.6, 135.8, 134.1, 134.2, 133.6, 133.5, 131.6, 131.4, 131.3, 129.8, 115.2, 115.0, 104.7, 104.3, 103.8, 103.9, 95.9, 96.0, 92.7, 92.8, 83.0, 83.2, 57.1, 57.0, 55.0, 52.6, 52.1, 52.0, 51.5, 51.5, 46.8, 47.4, 33.3, 33.5, 28.9, 29.5, 23.4, 23.3, 22.7, 22.6, 19.2, 19.3, 14.0, 14.1, 13.6, 13.5, 11.9, 11.8, 10.8, 10.7. EIMS (*m/z*): 725 (M⁺ + Na). HRMS: Calcd. For C₃₇H₄₁N₄O₁₀[M-H]⁺: 701.2823. Found: 701.2855.

3-Acetyl-15-glyoxilic acid-8-ketobacteriochlorin trimethyl ester 17

Compound **23** (15.0 mg, 0.02 mmol) was taken in a round bottom flask (100 ml) and dissolved in 15 ml conc. H₂SO₄. The reaction mixture was stirred vigorously at room temperature for 30 min and then poured into ice-water. Reaction mixture was then extracted with dichloromethane (100 ml), organic layer separated, washed with water, dried over sodium sulfate and concentrated. The residue obtained was purified with silica gel preparative plates using 2% acetone/dichloromethane to give **23** as a mixture of *cis*-diols (47:53). Yield: 5.5 mg (43%). UV-vis (THF, λ_{max}, nm, (ε): 403.1(7.68×10⁴), 425.0 (9.10×10⁴), 507.0 (1.17×10⁴), 539.0 (2.72×10³), 725.00 (8.70×10⁴), ¹H NMR (400 MHz, CDCl₃ δ 10.30 (s, 1H, meso-H), 9.44 (s, 1H, meso-H), 8.88 (s, 1H, meso-H), 4.48-4.51 (m, 1H, 8-H), 4.37 (s, 3H, CO₂Me), 4.24-4.28 (m, 1H, 7-H), 3.69 (s, 3H, CO₂Me), 3.68 (s, 3H, 12CH₃), 3.32 (s, 3H, 2-CH₃), 3.24 (s, 3H, COCH₃), 2.97-2.91 (m, 2H, 17¹-CH₂), 2.49 - 2.39 (m, 2H, 8-CH₂-CH₃ & 1H of 17²-CH₂), 2.09 - 2.15 (m, 2H, 8-CH₂-CH₃ & 17²-CH₂), 1.96 (d, 3H, 17-CH₃, *J* = 7.2 Hz), 1.86 (d, 3H, 7-CH₃, *J* = 7.2 Hz), 1.09-1.15 (m, 3H, 8-CH₂-CH₃), 1.50 (brs, 1H, NH), -1.6 (brs, 1H, NH). EIMS (*m/z*): 621.3 (M+Na).

Ring-D reduced methyl pheophorbide-a 24

Methyl bacteriopheophorbide **12** (20 mg, 0.032 mmol) was dissolved in dichloromethane (5 ml) under Ar atm. To this mixture, a solution of DDQ (5.9 mg, 0.026 mmol) in dry CH₂Cl₂ was added slowly. The resulting mixture was stirred at room temperature for 5 min and the entire reaction mixture was washed with water three times. The organic layer was separated and dried over anhydrous Na₂SO₄ and solvent was removed under vacuum. The residue obtained was purified with preparative plates using 3% acetone/ DCM affording 17 mg of the methyl pheophorbide-a. Yield: 17.0 mg (85%). UV-vis (CH₂Cl₂, λ_{max}, nm, (ε): 691 (3.45×10⁴). ¹H NMR δ (in CDCl₃): 9.95 (s, 1H, 5-H), 9.61 (s, 1H, 10-H), 8.77 (s, 1H, 20-H), 6.31 (s, 1H, 13²-H), 4.53 (m, 1H for 18-H), 4.25 (m, 1H for 17-H), 3.89 (s, 3H, 12-CH₃), 3.72 (s, 3H, 2-CH₃), 3.63 (s, 3H, 13²-COOCH₃), 3.57 (s, 3H, 17-CH₂CH₂COOCH₃), 3.27 (s, 6H, 7-CH₃ and 3-COCH₃), 2.62 (m, 2H, 17-CH₂CH₂COOCH₃), 2.28 (m, 4H, 2H of 17-CH₂CH₂COOCH₃, 2H of 8-CH₂CH₃), 1.83 (d, *J* = 7.6Hz, 3H, 18-CH₃), 1.70 (t, *J* = 7.2Hz, 3H, 8-CH₂CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 199.2, 189.5, 173.2, 169.4, 162.0, 153.2, 151.9, 148.8, 145.0, 139.1, 137.6, 135.9, 135.6, 134.4, 130.4, 129.8, 105.7, 104.0, 100.7, 94.2, 64.8, 52.9, 51.6, 51.4, 49.8, 33.4, 31.0, 29.8, 23.2, 19.4, 17.3, 13.8, 13.4, 12.2, 11.4, 11.2; EIMS (*m/z*): 623 (M+1). HRMS: C₃₆H₃₉N₄O₆[MH]⁺, Calcd 623.2870. Found: 623.2864.

Ring-B reduced methyl pheophorbide-a 25

Methyl bacteriopheophorbide **12** (20 mg, 0.032 mmol) was dissolved in dichloromethane (20 ml). To this mixture, a solution of FeCl₃·6H₂O (24 mg, 0.089 mmol) in nitromethane was added slowly. The resulting mixture was stirred at room temperature for 30 min, quenched by addition of 5 ml methanol, and washed with water three times. The organic

layer was separated and dried over anhydrous Na_2SO_4 and solvent was removed under vacuum. The residue obtained was purified with silica gel preparative plates using 2% acetone/dichloromethane. Yield: 16.0 mg (80%). UV-vis (CH_2Cl_2 , λ_{max} , nm, (ϵ): 682 (3.65×10^4). ^1H NMR δ (in CDCl_3): 9.51 (s, 1H, 5-H), 9.35 (s, 1H, 10-H), 8.77 (s, 1H, 20-H), 6.67 (s, 1H, 13²-H), 4.53 (m, 1H for 7-H), 4.30 (m, 1H for 8-H), 3.91 (m, 2H, 17- $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 3.81 (s, 3H, 12- CH_3), 3.72 (m, 6H, 3H of 18- CH_3 and 3H of 2- CH_3), 3.60 (s, 3H, 13²- COOCH_3), 3.29 (s, 3H, 17- $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 3.23 (s, 3H, 3- COCH_3), 2.90 (m, 2H of 8- CH_2CH_3), 2.08 (m, 2H, 17- $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 1.88 (d, $J = 7.2\text{Hz}$, 3H, 18- CH_3), 1.15 (t, $J = 7.2\text{Hz}$, 3H, 8- CH_2CH_3). ^{13}C NMR (100 MHz, CDCl_3): δ 203.3, 189, 172.9, 169.7, 166.4, 159.2, 155.4, 141.3, 140.2, 138.4, 133.3, 132.2, 127.9, 114.6, 113.3, 114.6, 100.1, 96.9, 96.2, 96.1, 66.2, 55.8, 53.1, 51.7, 48.3, 35.6, 33.3, 30.1, 29.2, 23.3, 22.1, 13.8, 11.9, 11.4, 10.8, 10.7; EIMS (m/z): 623 ($M+1$). HRMS: $\text{C}_{36}\text{H}_{39}\text{N}_4\text{O}_6[\text{MH}]^+$, Calcd 623.2870. Found: 623.2828.

In vitro photosensitizing efficacy

The photosensitizing activity of the compounds were determined as described before³⁰. The tumor cell lines used are Colon 26 (Mouse colon tumor). The Colon-26 tumor cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, L-glutamine, penicillin and streptomycin. Tumor cells were maintained in an atmosphere of 5% CO_2 , 95% air and 100% humidity at 37°C. For determining the PDT efficacy of the compounds, the cells were plated in 96 well plates at a cell density of 3000 cell/well in complete media. After 16 hrs of incubation at 37°C, the photosensitizers were added at variable concentrations and incubated at 37 °C for 24 hr in the dark. Prior to light treatment, the cells were replaced with drug-free complete media. Cells were then illuminated with light from an argon-pumped dye laser set at 725-741 nm respectively for each of the drugs as per their absorption wavelength in 17% bovine calf serum (BCS) measured before, at a dose rate of 1.6 mW/cm² for 0-2 J/cm². After PDT the cells were incubated for a further 48 h at 37 °C in the dark. Following the 48h incubation, 10 μL of 5.0 mg/mL solution of 3-[4,5-dimethylthiazol-2-yl]-2-5-diphenyltetrazoliumbromide (MTT) in PBS (Sigma, St. Louis, MO) was added to each well. After 4 h incubation at 37°C, the MTT and the media were removed, and 100 μL of DMSO was added to solubilize the formazan crystals. The 96- well plate was read on a microtiter plate reader (BioTek Instruments, Inc., ELx800 Absorbance Microplate Reader) at an absorbance of 570 nm. The results were plotted as a percent survival of the corresponding dark (drug no light) control for each compound tested. Each data point represents the mean from two separate experiments, with 6 replicate wells and the error bars are the standard deviation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Scheme 1.
Conversion of chlorins to ketobacteriochlorins

Scheme 2.

Regioselective synthesis of ring-D and ring-B chlorins (**5** and **7** respectively) from bacteriochlorin **6**.

Scheme 3.

Synthesis of ring-B and ring-D reduced ketobacteriochlorins from ring-B and ring-D reduced chlorins.

Scheme 4.
Synthesis of rhodobacteriochlorin **14** and the corresponding 18-keto-bacteriochlorin **16**.

Scheme 5.
Synthesis of 7-keto and 8-keto-ring-D reduced and 18-keto-ring-B reduced bacteriochlorins.

Scheme 6.

Ring-B and ring-D reduced chlorins derived from methyl bacteriopheophorbide-a on reacting with $\text{OsO}_4/\text{H}_2\text{SO}_4$ gave complex mixture.

Scheme 7.

The proposed mechanism for the formation ring-B and ring D reduced ketobacteriochlorins obtained from their corresponding diols under Pinacol-Pinacolone reaction conditions.

Figure 1.
HPLC chromatogram of bacteriochlorin **11**, **17**, **20** and **21** (for details see the Supporting Material Information)

Figure 2.

Partial ^1H -NMR of ketobacteriochlorins **11**, **17**, **20** and **21** (only *meso*- regions are shown).

For details see the “Experimental” and “Supporting Information” parts of the paper.

Figure 3.
NOE correlations of ketobacteriochlorins **11**, **17** and **21**.

Compounds	9	9a	11	17	20	21
Absorbance	725	714	715	731	737	732
Fluorescence Emission	736	727	732	735	765	741
Stokes shift (nm)	11	13	17	4	28	9

Figure 4. Electronic absorption spectra and fluorescence emission spectra of keto-bacteriochlorins **9**, **9a**, **11**, **17**, **20** & **21** at equimolar concentration (1.2 μM in CH_2Cl_2).

Figure 5.

(A) *In vitro* photosensitizing efficacy of bacteriochlorins **9**, **9a** (containing a keto- group in ring-B), **11** (containing a keto- group in ring-D), **17**, and **20/21** isomeric mixture (bearing keto-groups in ring-B) at variable concentrations and light doses. The Colon 26 tumor cells were exposed to light at 1 J/cm^2 at 24 h post-incubation and the MTT assay was performed after 48 h. (B) *In vitro* dark toxicity of photosensitizers **9**, **9a**, **11**, **17**, **20/21** incubated in Colon26 tumor cells for 24 h, but did not exposed to light. (C) The inset figure shows the PDT efficacy of the separated isomers **20** and **21** at similar concentrations as the parent mixture and exposed to light (dose: 1 J/cm^2).