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Thermolysis of *vic*-Dihydroxybacteriochlorins: Effect of the Nature of Substrates in Directing the Formation of Chlorin−Chlorin Dimers with Fixed and Flexible Orientations and Their Preliminary in Vitro Photosensitizing Efficacy†

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Received December 26, 2002

The thermolysis products obtained by refluxing a series of *vic*-dihydroxychlorins in *o*-dichlorobenzene are characterized. Depending on the nature of substrates, this methodology provides an access for novel carbon−carbon linked chlorin−chlorin dimers and chlorin−porphyrin dimers with fixed and flexible orientations**.** The configuration of the linkers in the symmetrical and unsymmetrical dimers was confirmed by extensive NMR (COSY, ROESY) and molecular modeling studies. The molecular modeling studies of the energy-optimized dimers with flexible orientation confirmed that one of the chlorin units of the dimeric structure is tilted toward the opposite ring as evident by the shielding effect in the resonances of some of the protons in the ^{1}H NMR spectroscopy. Among the dimers with fixed orientation, compared to the free-base analogues, the related mono- and di-Zn(II) complexes produced a decreased fluorescence intensity, suggesting a possibility of the faster energy transfer via intersystem crossing (ISC) in the metalated derivatives than the corresponding free-base analogues to produce the corresponding excited triplet states. The photosensitizing efficacy of the monomers and the related dimers was also compared in radiation-induced fibrosarcoma (RIF) tumor cells at variable drug/light doses. In preliminary screening, compared to monomers, the corresponding carbon−carbon linked dimers produced enhanced photosensitizing efficacy.

Introduction

Porphyrin dimers and higher oligomers have attracted significant attention due to their potential use in the bacterial photosynthetic reaction center.[1]In recent years, contributions from several research groups have resulted in the synthesis of a variety of novel structures in which arrays of porphyrins or porphyrin-based compounds are linked by rigid or flexible spacers.[2] It has been shown that close proximity among the porphyrins plays an important role for efficient energy transfer.[3] Distance, geometry, and orientation have been recognized as important factors for control of the efficiency of the energy- and electron-transfer process.[4] Besides their application as models to understand the photosynthetic reaction centers, certain carbon−carbon linked porphyrin-based dimers and higher oligomers are also reported as effective photosensitizers for photodynamic therapy (PDT).[5] Among the components of Photofrin (a porphyrin-based photosensitizer containing a mixture of monomers, dimers, and higher oligomers linked with ether, ester, and/or carbon−carbon linkages), compared to monomers the corresponding dimers with ether[6] and carbon−carbon

† A part of this work was published as a communication: Kozyrev, A. N.; Zheng, G.; Shibata, M.; Alderfer, J. L.; Dougherty, T. J.; Pandey, R. K. *Org. Lett*. **1999**, *1*, 1193.

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10.1021/jo0207630 CCC: $25.00
Published on Web 04/12/2003

bridges[7] were found to be more effective both in vitro and in vivo photosensitizing efficacy. However, the related dimers and higher oligomers with ester linkages[8] were found to be unstable and did not produce any significant photosensitizing activity.

For quite some time, one of the objectives of our laboratory has been to synthesize stable bacteriochlorin-based photosensitizers for use in PDT.[9] Due to their long-wavelength absorption in the near-IR region, these compounds could be useful in treating tumors that are deeply seated. Therefore, for the synthesis of such a system, our first objective was to develop an efficient methodology for the preparation of 8-vinyl chlorins which on being subjected to Diels−Alder reactions could be converted into the related bacteriochlorins by following the well-established methodology for the preparation of benzoporphyrin derivatives and the related analogues. In our previous study, we have shown that under appropriate conditions, the *vic*-dihydroxybacteriopurpurin-18 methyl ester can be converted into the corresponding 8-vinylpurpurin-18 methyl ester, which on further reaction with dimethyl acetylenedicarboxylate produced the corresponding benzobacteriopurpurin-18 methyl ester that has a long-wavelength absorption near 800 nm.[10] Unfortunately, the anhydride ring system present in the molecule was found to be unstable in vivo and the resulting product was identified as the related bacteriochlorin p_6 with a significant blue-shift in its electronic absorption spectrum[11] and the characteristics of the parent chromophore were lost. Therefore, it was decided to extend this methodology by preparing certain stable 8-vinylchlorins, converting them into the corresponding bacteriochlorins, and comparing their biological efficacy with that of known stable bacteriochlorin systems. This paper describes the isolation, characterization, and spectroscopic properties of a series of thermolysis products (including the desired 8-vinylchlorins) obtained by thermolysis of various *vic*-dihydroxybacteriochlorins which in turn were obtained in a sequence of reactions from chlorophyll *a*. Starting from 8-vinylchlorins, we have prepared a series of the related benzobacteriochlorin analogues. The synthesis of these novel structures and their detailed in vitro/in vivo biological efficacy will be published elsewhere.

Results and Discussion

For our initial study, readily available mesochlorin e_6 trimethyl ester **1** and methyl mesopyropheophorbide *a* **2** were used as substrates and individually reacted with OsO_4/H_2S to produce the corresponding *vic*-dihydroxychlorins **3** and **4**.[12] Several reaction conditions were used for converting the chlorin diols **3** and **4** to the related 8-vinyl analogues.[13] However, the best results were obtained by refluxing bacteriochlorin **3** in *o*-dichlorobenzene for 1.5 h and the 8-vinylchlorin **5** was isolated in 41% yield. The other reaction products were identified as 8-keto-bacteriochlorin **6** (7%), bis-chlorin dimer **7** linked with an ethylene group at position-7 (18%), and dimer **8** containing a methyl-vinyl joining the two chlorin units (4%). The structures of the monomers and the dimers were confirmed by NMR (H−H COSY, ROESY) and mass spectrometry analyses. The FAB-MS of compound **7** showed a molecular ion peak at 1277.5 (MH^+), indicating a dimeric structure. The appearance of a single set of resonances in the NMR spectrum for both the chlorin molecules suggested a possibility of the presence of symmetry between the two chlorin units. The resonances for the meso protons were observed as singlets at δ 9.96, 9.91, and 8.70 ppm, each integrating for two protons. The fourth singlet appeared at δ 8.84 ppm and was assigned to the protons of the newly formed symmetrical CH═CH bond bridging the two chlorin moieties. In its ROESY spectrum, the strong correlations of the proton at δ 8.84 (1H, s, CH═CH) with H-5 (δ 9.96, s) and CH_3CH_2-8 [δ 4.33 (2H, q, J = 7.6 Hz), 2.13 (3H, t, J = 7.6 Hz)] indicated that the two chlorin units were joined together at position-7. The configuration of the double bond was confirmed to be *trans*, since no upfield shift effects common in such cofacial porphyrin and chlorin systems (obtained by reacting the formyl derivatives under McMurry reaction conditions and then refluxing the respective intermediates with acetic acid[14]) for the −NH and the exocyclic ring protons were observed. The structure of dimer **8** containing a methylvinyl group as a linker was found to be similar to that obtained previously from *vic*-dihydroxy-mesopurpurin-18-*N*-hexylimide.[15]

Replacing **3** with *vic*-dihydroxy-methyl-mesopyropheophorbide **4** produced mainly 8-vinylchlorin **10** (54%) and the related bis-dimer containing a methylvinyl linker **11** (9%). Interestingly, the symmetrical dimer **14** with an ethylene bridge, analogous to the product **7** isolated in our chlorin e_6, study was not observed. Therefore, the formation of thermolysis products seems to depend on the nature of the substrates. This methodology was further extended in other chlorin systems, mesochlorin p_6 trimethyl ester **15**, mesopurpurin-18-*N*-hexylimide **16**, and mesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide **17**. Reaction of these individual chlorins with osmium tetraoxide and subsequent treatment with refluxing *o*-dichlorobenzene gave the corresponding 8-vinylchlorins (**21**, **25**, **28**) and 8-keto bacteriochlorins (**22**, **26**, **29**). In addition, except chlorin p_6, which gave both dimers **23** and **24**, chlorins **16** and **17** afforded only one class of related dimers **27** and **30** with flexible orientation.

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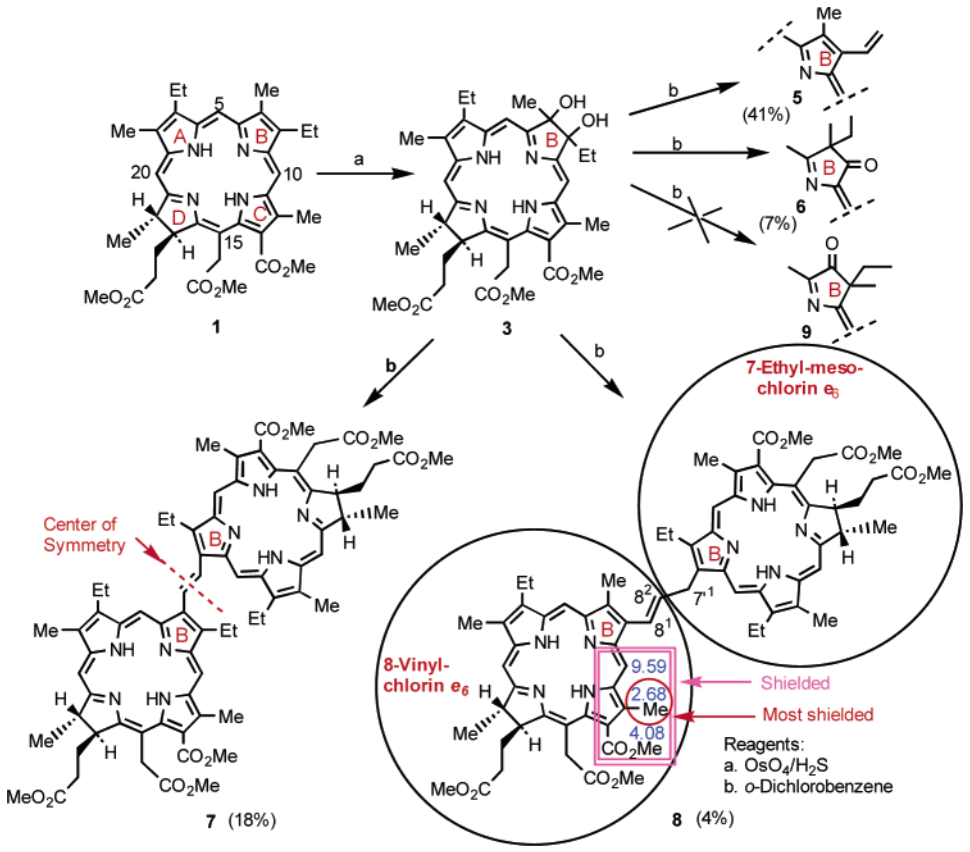
SCHEME 1. Thermolysis Products Isolated from *vic*-Dihydroxymesochlorin e_6 Trimethyl Ester 3



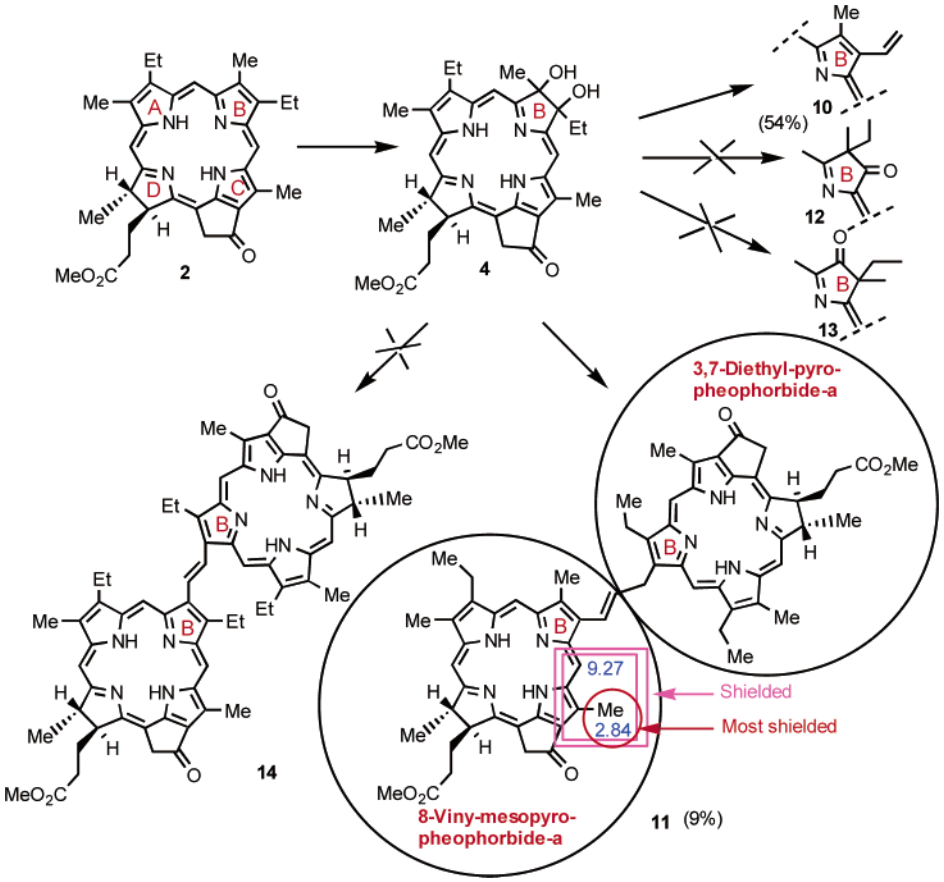
To investigate the rate of energy transfer between the metalated and the related free base chromophores, bis-dimer **7** with a fixed orientation on reacting with Zn(II) acetate produced a mixture of the related mono- and di-Zn(II) complexes **31** and **32**, respectively (Scheme 4). The ^{1}H NMR spectrum of mono-Zn dimer **31** exhibited two sets of chlorin signals that further confirmed its dimeric structure. A complete ^{1}H NMR assignment for the individual protons was achieved by 2D NMR (H−H COSY and ROESY) studies.

The mechanism of the formation of all the monomeric and dimeric structures seems to depend on the nature of various intermediate carbocations. In contrast to the pinacol−pinacolone rearrangement of the chlorin diols, which generally produce both 7- and 8-ketochlorins, under thermolysis conditions only 8-ketochlorins were observed. These results indicate that the hydroxyl function at position-7 is leaving first to produce the carbocation **33**, which quickly rearranges to afford the corresponding 8-ketochlorins. A possible mechanism for the formation of dimers with vinyl- and vinyl-methyl linkages and other dehydration products is shown in Scheme 5. The formation of certain proposed intermediate carbocation species [e.g. heterolysis of a C−OH (**33**)] should be facilitated in the presence of acid. Though all reactions were performed in freshly distilled *o*-dichlorobenzene, the presence of traces of acid impurities cannot be ruled out. However, reaction of *vic*-dihydroxybacteteriochlorins under strong acidic conditions mainly produced the corresponding 7-keto- and/or 8-keto-bacteriochlorins, without formation of any related 8-vinylchlorins or the dimeric structures.

For the formation of dimers with vinyl-methyl linkers, the proposed mechanism indicated that the carbocation **35b** might be a more stable reactive intermediate than others, and if an aromatic compound containing a vinyl substituent is added in large excess, we might be able to "trap" this particular intermediate, which could produce a heterodimeric structure. To confirm this hypothesis, 3-fold excess of rhodoporphyrin dimethyl ester **38** was reacted with bacteriochlorin **19**. As hypothesized, along with 7-keto derivartive **26** (10%) and 8-vinylpurpurin-18-*N*-hexylimide **25** (42%), the novel porphyrin−chlorin dimer **39** was isolated in 31% yield (Scheme 5) without the formation of any homodimer. The UV/visible spectrum of heterodimer **39** exhibited distinctive structural features for both porphyrin and chlorin subunits. In the NMR spectrum of dimer **39**, the resonances of one of the *meso*-hydrogen and the 7-methyl protons of the vinylporphyrin showed upfield shifts ($\Delta\delta = -0.28$ and -0.7 ppm, respectively). These data indicate that similar to other dimers **8**, **11**, **24**, **27**, and **30** in heterodimer **39** the porphyrin unit is oriented in a tilted fashion above the chlorin plane.

Molecular Modeling. The NMR data of a series of dimers with a vinyl-methyl linker, derived from various chlorins, showed a similar pattern. For example, the protons correspond to 12-methyl, and 10-*meso*-H of one of the monomers of the dimeric structure showed a significant upfield shift in the NMR spectrum, possibly due to the shielding effect caused by another chlorin unit. To obtain possible dimeric structures that are consistent with the observed NMR data, in our initial study, dimers **7** and **8** obtained from mesochlorin e_6 were selected for

SCHEME 2. Thermolysis Products Isolated from *vic*-Dihydroxymesopyropheophorbide *a* 4



the molecular modeling analysis. The structures of compounds **7** and **8** were built with a combination of quantum mechanics and molecular mechanics molecular modeling techniques. Since there is no crystal structure of the corresponding dimers available, first, an appropriate template for the monomer units of the above compounds was searched on the Cambridge Structural Database (V5.2.3) with ConQuest software (V1.4).[16] The crystal structure of 3^1,3^2-didehydrorhodochlorin-15-acetic acid trimethyl ester (CSD code ZUBBIH)[17] was selected as a template for dimers **7** and **8** based on structural similarity. After appropriate modifications of the ring substituents by the SYBYL molecular modeling program version 6.8 (Tripos Inc., St. Louis, MO) with the standard geometry and the SYBYL fragment library, the geometry of the monomer unit was fully optimized with a semiempirical molecular orbital method, PM3, with the SPARTAN (V5.1.3) program (Wavefunction Inc, Irvine, CA). With use of the resulting optimized geometry, the dimer structures were modeled by adding two different kinds of linkers with appropriate modifications by the SYBYL software. The extended conformation was assumed for the linker region. The resulting geometry was again subject to energy optimization with a semiempirical MO, PM3. Compounds **7** showed a limited flexibility at the linker region where only two possible conformations exist for this dimer and were built by adjusting appropriate torsional angles and subjected to energy optimization with a semiempirical MO, PM3. Both conformers had very similar energy and one of the PM3 optimized structures for these dimers is shown in Figure 1A. Essentially the extended flat structures were obtained in both cases.

On the other hand, the linker for the related dimer with a vinyl-methyl linkage **8** possesses a large flexibility and many conformations are possible for this dimeric structure. The conformational search was performed by using the SYBYL software random search module with the standard Tripos force field and the Del Re σ and Hückel π charges. Since the modifications of torsions in dimer linker regions may force changes to other exocyclic (or ring) substituent conformations, all flexible torsional variables were included in the conformational search calculations. The search produced many conformations and each structure was examined first graphically to select candidate structures that best correspond to the NMR data. The interactions of the C12 methyl and C10 hydrogen groups with the aromatic ring from another monomer unit were used as the main criteria by measuring the distances between hydrogens and the plane defined by aromatic rings. The possible candidate selected from the empirical conformational search calculations was subjected to the subsequent energy optimization by a semiempirical MO, PM3 (Figure 1B).

In the 3D structures of the dimer **39** with a methyl-vinyl linker, the two chromophores showed a "tilted" arrangement, similar to the geometrical arrangement of bacteriochlorophyll B_A and bacteriochlorophyll F_A in the bacterial photosynthetic center (RC) of *Rb. sphaeroides*. In comparison to the natural system in which the center-

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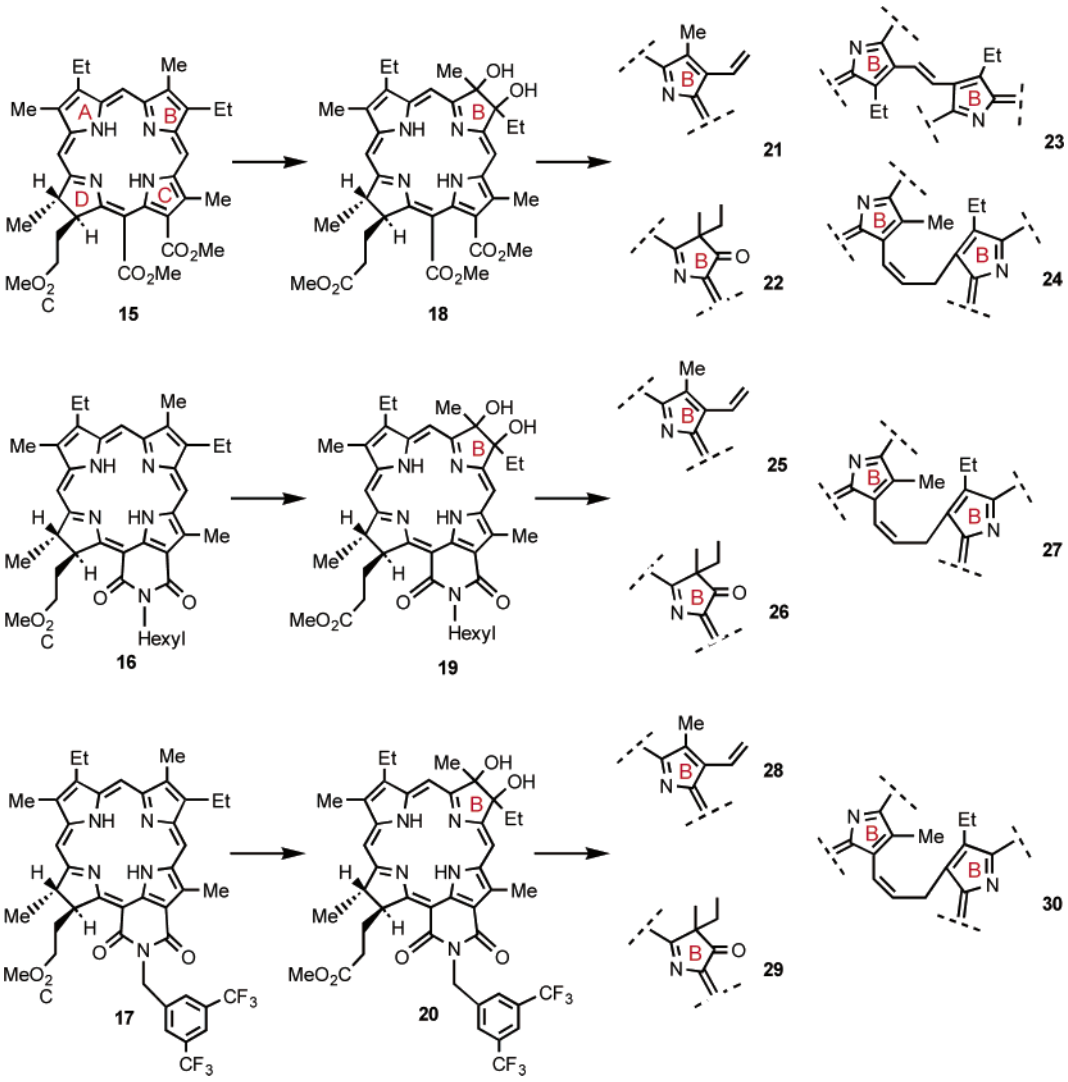
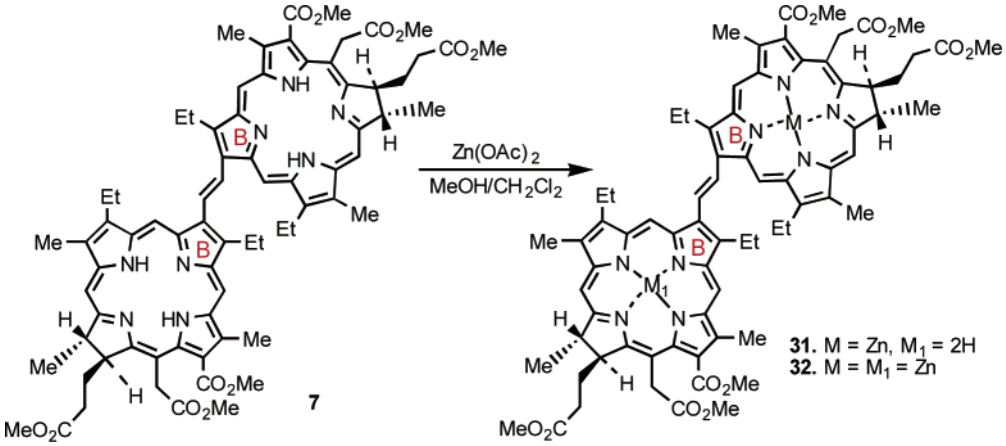
SCHEME 3. Thermolysis Products Isolated from from *vic*-Dihydroxy Analogs of Mesochlorin p_6 and *N*-Substituted Mesopurpurinimides



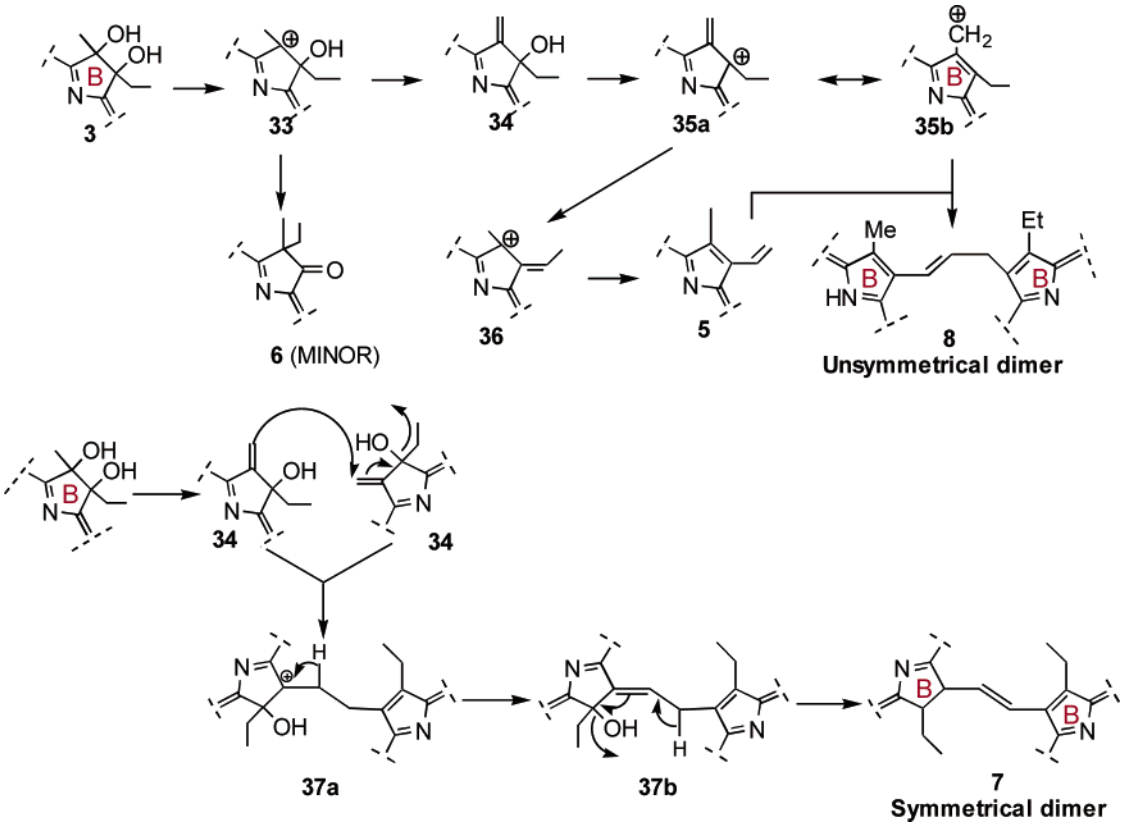
SCHEME 4. Conversion of the Free-Base Dimer 7 into the Corresponding Mono- and Di-Zn(II) Complexes 31 and 32, Respectively



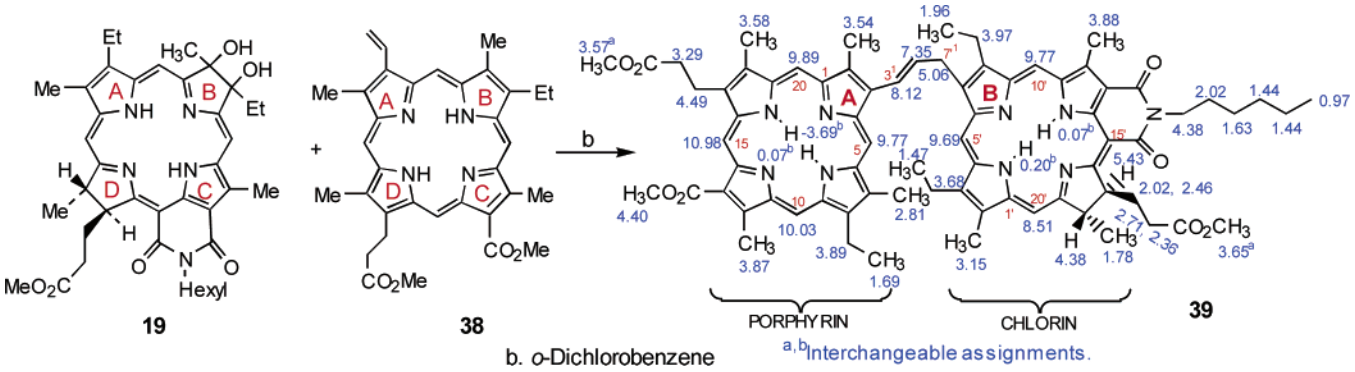
to-center distance was calculated to be 11.7 Å, the corresponding distance for dimer **39** was found to be 9.3 Å. Further, the 12-methyl substituent in dimer **39** is arranged in an identical fashion as observed for the methyl group for bacteriopheophytin *a* of the F_A-F_B pair.[18] These data indicate that these dimers could be interesting models for studying ET processes.

Photophysical Properties. The electronic absorption spectra of the free-base dimer **7** and the corresponding mono- and di-Zn(II) complexes were measured in dichlo-

SCHEME 5. Possible Mechanisms for the Formation of Symmetrical and Unsymmetrical Dimers



SCHEME 6. Synthesis of Porphyrin−Chlorin Dimer 39



romethane at equimolar concentrations and were found to be quite different. The Qy transition of the free-base **7** was observed at 653 nm while the corresponding di-Zn(II) complex **32** was observed at 627 nm with a significant decrease in "Soret" band intensity. The related mono-metalated analogue **31** showed characteristic absorption for both the chromophores (Figure 1). Excitation of **7**, **31**, and **32** at 416, 414, and 411 nm gave a single fluorescence emission band at 659, 658, and 636 nm, respectively, suggesting that the conjugated system of the two macrocycles is strongly coupled. Compared to the free-base analogue the corresponding mono- and di-metalated derivatives produced a decreased fluorescence intensity (**7** > **31** > **32**) [Figure 2]. These results suggest the possibility of a faster energy transfer via intersystem crossing (ISC) in the metalated analogues than the free-base to produce the corresponding excited triplet states. A detailed photophysical study with these and other related dimers is currently in progress.

In Vitro Photosensitizing Activity. The photosensitizing efficacy of a series of monomers **1**, **2**, **5**, **10**, **15**, **16**, **17**, **21**, **25**, and **28** and dimers with carbon−carbon linkages **7**, **8**, **11**, **23**, **24**, **27**, and **30** was compared in RIF tumor cells at two different drug and variable light doses. Viability was measured by trypane blue exclusion.[19] The results are summarized in Figure 4 (parts A, B, C, D). As can be seen from Figure 4 (parts A and B), the monomers and dimers at a drug concentration of 1.25 μM (except monomers **25** and **28** and dimer **7**) showed

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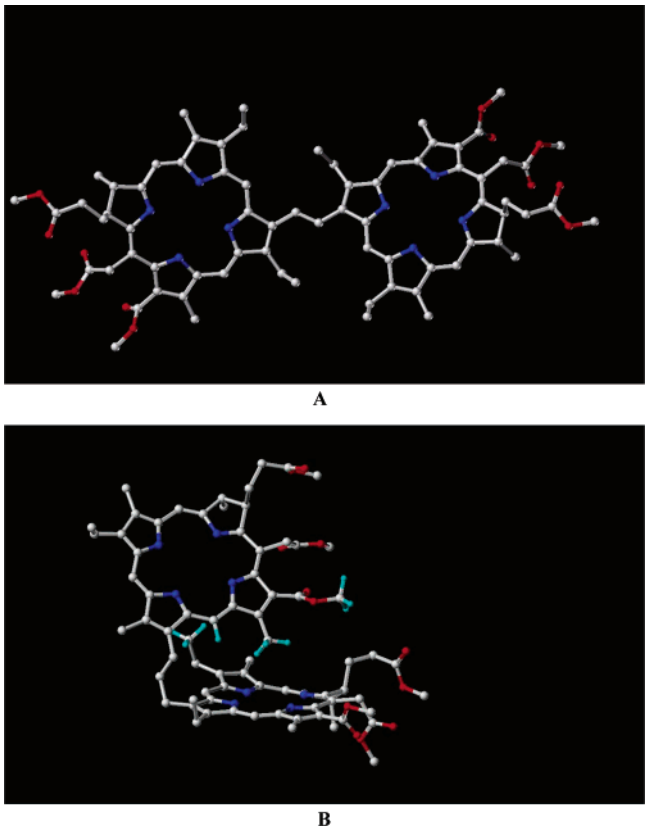

FIGURE 1. The 3D structure of the energy-optimized mesochlorin e_6 dimers: (A) dimer **7**, two molecules are joined with an ethylene bridge at the 7−7′ positions (trans configuration); (B) dimer **8**, two chlorin units are joined at the 8−7′ positions with a methylvinyl linker. The shielded protons pointing toward the opposite ring are drawn in cyan color (see the text).

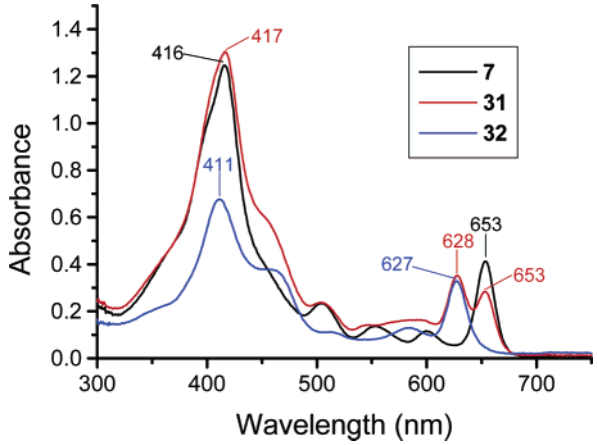

FIGURE 2. The electronic absorption spectra of dimers **7**, **31**, and **32** at equimolar concentration (5.0 μM) in dichloromethane.

good efficacy. However, among the most effective compounds it was difficult to establish a correlation between the monomeric and the related dimeric structures. Therefore, all monomers and dimers were also evaluated at a lower concentration (0.6 μM). From the results summarized in parts C and D of Figure 4, it is evident that compared to monomers all the dimers showed a significant increase in PDT efficacy. For example, at a lower concentration, none of the monomers showed any significant photosensitizing efficacy whereas the related carbon−carbon dimers showed 20−50% cell kill at the light dose of 5 J/cm^2. However, on the basis of preliminary in vitro studies it is very difficult to predict the in vivo outcome, because the rate of the clearance of the drug plays an important role in tumor localization, which generally has a great impact in in vivo efficacy.

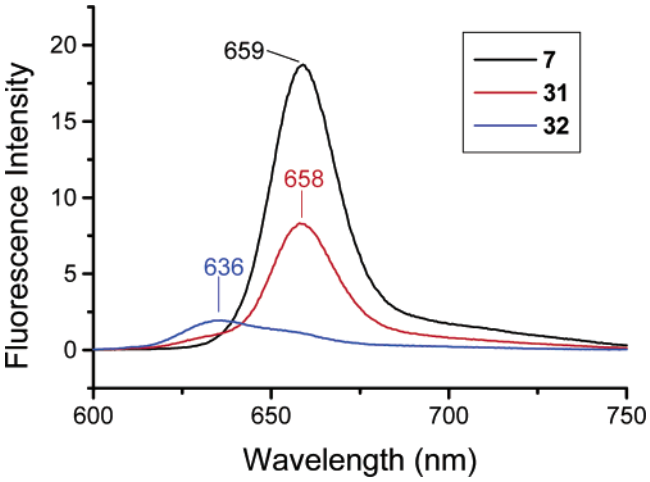

FIGURE 3. The emission spectra of dimers **7**, **31**, and **32** (concentration: 1.0 μM) in dichloromethane. See Table 3.

In conclusion, the results obtained from our present study clearly indicate that the nature of formation of the thermolysis products from *vic*-dihydroxybacteriochlorins depends on the stability of the intermediate carbocations, which are found to be substrate dependent. The utility of this approach for the preparation of new free-base dimers derived from chlorin e_6 and purpurin p_6 linked at position 7 of the monomeric units with fixed orientation is also presented. We have also shown the utility of this approach in constructing unsymmetrical structures as models for "twisted" intramolecular charge-transfer systems. In these models, the connecting bridge has slight flexibility to allow minimum overlap between the chromophore π systems. In a comparative in vitro study, both symmetrical and unsymmetrical dimers produced improved photosensitizing ability over the related monomeric structures. The detailed photophysical and in vivo photosensitizing efficacy, including pharmacokinetic and pharmacodynamic properties of these and other related chlorin−chlorin, bacteriochlorin−bacteriochlorin dimers with carbon−carbon or ether linkages are currently in progress and will be published elsewhere.

Experimental Section

^{1}H NMR spectra were recorded in $CDCl_3$ solutions at 400 or 600 MHz. Chemical shifts are reported in ppm with residual $CHCl_3$ in $CDCl_3$ as internal standard (for ^{1}H, 7.26 ppm). 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^2 TLC plates (Analtech).

General Procedure for Thermolysis of *vic*-7,8-Dihydroxychlorins. A solution of *vic*-7,8-dihydroxychlorins (243−491 mg) in *o*-dichlorobenzene (30−40 mL) was heated under reflux in an atmosphere of nitrogen for 1.5 h. After being cooled to room temperature, the reaction mixture was loaded on a short silica column, eluted with hexanes to remove *o*-dichlo-

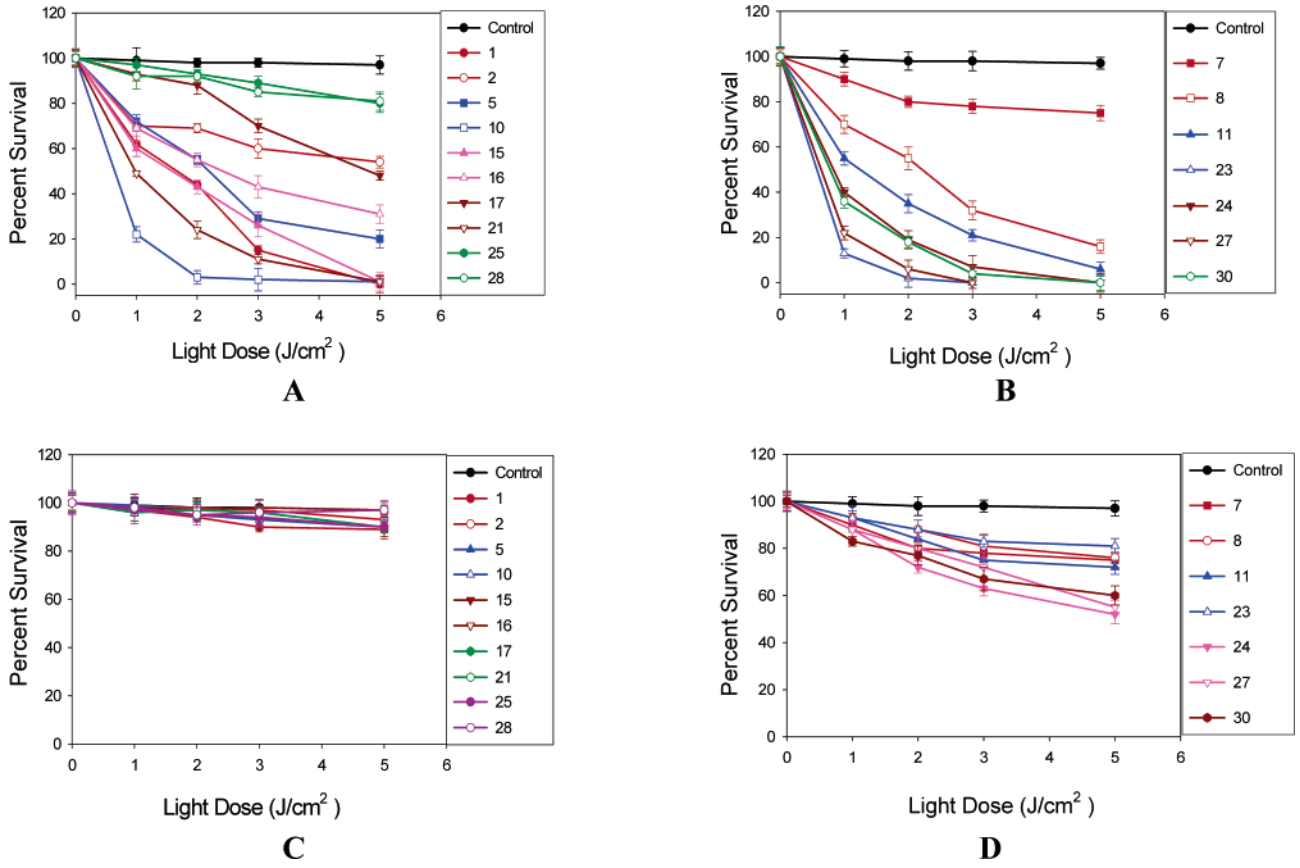

FIGURE 4. In vitro photosensitizing efficacy: A and B, monomers and dimers at the concentration of 1.25 mM; C and D, monomers and dimers at the concentration of 0.625 mM in RIF tumor cells incubated for 3 h (48 h MTT).

robenzene, and then eluted with 10% MeOH/CH_2Cl_2. The residue obtained after removing the solvents was separated by silica gel chromatography and preparative silica plates. Depending on the substrate, the related 8-vinylchlorins, 8-ketobacteriochlorins, and symmetrical and/or unsymmetrical dimers were obtained in variable yields. The experimental details of all the vinyl analogues are described in a separate paper that also includes the syntheses and biological efficacy (in vitro/in vivo) of the corresponding Diels−Alder products (Pandey and co-workers, unpublished results).

Thermolysis Products of *vic*-7,8-Dihydroxymesochlorin e_6 Trimethyl Ester 3 (5, 6, 7, and 8). Diol **3** (491 mg) was treated with *o*-dichlorobenzene (40 mL) by following the procedure as described above (general procedure). The purification was carried out with silica gel column chromatography, eluting with a dichloromethane/acetone mixture (v/v 15/1) to give the 8-vinylchlorin **5** (192 mg, 41%). Further eluting with 10% MeOH/CH_2Cl_2 gave a mixture. The mixture was further purified by preparative silica gel TLC plates, using dichloromethane/acetone (v/v 15/1) as eluent. Three major bands were collected from the plates. The first band (faster moving) was identified as 8-ketobacteriochlorin **6** (33 mg, 7%). The second band was identified as symmetrical dimer **7** (84 mg, 18%), and the most polar band was identified as unsymmetrical dimer **8** (18 mg, 4%). Spectral data of **6**: UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 396 (90782), 414 (77118), 496 (8985), 529 (4024), 648 (9546), 685 (26299). ^{1}H NMR data (see Table 1). MS (FAB) *m/z* 656.4 (M^+, 100). HRMS (FAB): Calcd for $C_{37}H_{44}N_4O_7$ 656.3210; found 656.3210. Spectral data for **7**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 416 (241908), 504 (45332), 553 (26581), 600 (22460), 653 (80115). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.96 (1H, s, H-5), 9.91 (1H, s, H-10), 8.84 (1H, s, H-7^1), 8.70 (1H, s, H-20), 5.32 (2H, AB system, J = 19.0 Hz, CH_3OOCCH_2−), 4.45 (2H, m, H-17 and H-18), 4.33 (2H, q, J = 7.6 Hz, CH_3CH_2-8), 4.30 (3H, s, CH_3OOC−), 3.88 (2H, q, J = 7.5 Hz, CH_3CH_2-3), 3.81 (3H, s, CH_3OOC−), 3.66 (6H, s, CH_3-12 and CH_3OOC−), 3.36 (3H, s, CH_3-2), 2.58, 2.24, 1.82 (1H, 2H, 1H, m, $CH_3OOCCH_2CH_2$-17), 2.13 (3H, t, J = 7.6 Hz, CH_3-CH_2-8), 1.80 (3H, d, J = 7.1 Hz, CH_3-18), 1.74 (3H, t, J = 7.8 Hz, CH_3CH_2-3), −1.05 (1H, br, −*NH*), −1.11 (1H, br, -*NH*). MS (FAB) *m/z* 1277.8 (MH^+, 100). HRMS (FAB): calcd for $C_{74}H_{85}N_8O_{12}$ 1277.6286; found 1277.6310. Spectral data for **8**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 409 (225784), 500 (24531), 597 (9813), 650 (67115). ^{1}H NMR data (see Table 2). MS (FAB) *m/z* 1277.8 (MH^+, 100). HRMS (FAB): calcd for $C_{74}H_{84}N_8O_{12}$ 1277.6286; found 1277.6280.

Thermolysis of *vic*-7,8-Dihydroxymethylmesopyropheophorbide a 4 (10 and 11). Diol **4** (364 mg) was treated with *o*-dichlorobenzene (30 mL) as described in the general procedure. The purification was carried out with silica gel column by eluting with 3% methanol/dichloromethane to give the 8-vinylchlorin **10** (184 mg, 54%), then eluting with 10% MeOH/CH_2Cl_2 to give a mixture. The mixture was further separated by preparative silica gel TLC plates, developed with hexanes/dichloromethane/ethyl acetate (v/v/v, 1/1/2). The unsymmetrical dimer **11** (31 mg, 9%) was obtained. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 318 (46273), 399 (163952), 419 (205305), 506 (21940), 538 (15159), 601 (17552), 656 (84569). ^{1}H NMR data (see Table 2). MS (FAB) *m/z* 1277.8 (MH^+, 100). HRMS (FAB): calcd for $C_{68}H_{73}N_8O_6$ 1097.5653; found 1097.5680.

Thermolysis of *vic*-7,8-Dihydroxymesochlorin p_6 Trimethyl Ester 18 (21−24). Diol **18** (342 mg) was treated with *o*-dichlorobenzene (30 mL) as described in the general procedure. The purification was carried out with a silica gel column by eluting with dichloromethane/ethyl acetate (v/v 10/1) to give the 8-vinylchlorin **21** (128 mg, 40%), then eluting with 10% MeOH/CH_2Cl_2 to give a mixture. The mixture was further purified by preparative silica gel TLC plates [solvent system: hexanes/dichloromethane/ethyl acetate (v/v/v, 8/8/9)]. Three major bands were collected from the plates. The first band was

TABLE 1. ^{1}H NMR Spectral Data of 8-Keto-bacteriochlorins 6, 22, 26, and 29[a]

	6	22	26	29
2-CH_3	3.19, s	3.16, s	3.15, s	3.13, s
3-CH_2CH_3	3.65, m	3.62, q, J = 7.4	3.61,[d] q	3.59, q, J = 7.7
3-CH_2CH_3	1.66, t, J = 7.1	1.65, t, J = 7.6	1.64, t, J = 7.6	1.64, t, J = 7.6
5-H	8.30, s	8.27, s	8.26, s	8.24, s
7-CH_3	1.82, s	1.84 and 1.80, s	1.81, splitting s	1.81 and 1.79, s
7-CH_2CH_3	2.54, m	2.53, m	2.50, m	2.51 and 2.42, m
7-CH_2CH_3	0.50, t, J = 7.6	0.54, t, J = 7.2	0.54, t, J = 7.5	0.54, t, J = 7.2
10-H	9.31, s	9.33, s	9.33, s	9.30, splitting s
12-CH_3	3.37, s	3.46, s	3.66, s	3.64, s
15-$CH_2CO_2CH_3$	5.08, AB system, J = 18.9			
17-H	4.16, m	4.92, br d, J = 8.5	5.23, d, J = 8.0	5.16, br d, J = 8.8
17-$CH_2CH_2CO_2CH_3$	2.54 (1H), 2.20 (2H), 1.75 (2H), all m	2.37 (1H), 2.12 (2H), 1.85 (1H), all m	2.67 (1H), 2.37 (2H), 1.95 (1H), all m	2.67, 2.39, 2.30, 1.91, all 1H, m
18-H	4.16, m	4.16[c]	4.19, m	4.18, q, J = 7.4
18-CH_3	1.67, d, J = 6.8	1.78, d, J = 7.5	1.69, d, J = 7.3	1.69, d, J = 7.3
20-H	8.28, s	8.23, s	8.25, s	8.22, s
$-CO_2CH_3$	3.65, 3.76, 4.20, all s	3.56, 4.12, 4.17, all s	3.59, s	3.58, splitting s
−NH	0.20, br s	0.51, br s	0.84, br s	1.08, s
−NH	−0.12, br s	0.26, br s	0.48, br s	0.69, s
$-NCH_2C_6H_3(CF_3)_2$[e]	8.18 (2H, s), 7.79 (1H, s), 5.71 (2H, s)			
$-NCH_2(CH_2)_4CH_3$[f]	4.40 (2H, m), 1.95 (2H, m), 1.59 (2H, m),[b] 1.43 (4H, m), 0.94 (3H, t, J = 7.5)			

[a] All NMR spectra were recorded at 400 MHz. The coupling constants (J) are expressed in hertz. [b] Overlapped with 3-CH_2CH_3 and water signals. [c] Overlapped with a methyl resonance. [d] Overlapped with 12-CH_3. [e] For compound **29** only. [f] For compound **26** only.

identified as 8-ketobacteriochlorin **22** (41 mg, 12%). The second band was identified as symmetrical dimer **23** (10 mg, 3%), and the third band was identified as unsymmetrical dimer **24** (29 mg, 9%). Spectral data for **22**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 389 (107881), 469 (5080), 496 (9605), 529 (7462), 633 (10002), 690 (29848). ^{1}H NMR data (see Table 1). MS (FAB) m/z 642.4 (M^+, 100). HRMS (FAB): calcd for $C_{36}H_{42}N_4O_7$ 642.3053; found 642.3076. Spectral data for **23**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 412 (215152), 501 (40152), 555 (20455), 605 (16667), 656 (71212). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.91, 9.86 (each 1H, s, H-5 and H-10), 8.73, 8.62 (each 1H, s, H-7^1 and H-20), 5.19 (1H, dd, J = 9.0, 2.4 Hz, H-17), 4.41 (1H, q, J = 7.4 Hz, H-18), 4.27 (2H, q, CH_3CH_2-8, overlapped with a methyl ester signal), 4.26 (3H, s, CH_3OOC−), 4.20 (3H, s, CH_3OOC−), 3.82 (2H, q, J = 7.6 Hz, CH_3CH_2-3), 3.73, 3.55, and 3.31 (each 3H, s, CH_3-2, CH_3-12, and CH_3OOC−), 2.41, 2.25, 2.11, and 1.90 (each 1H, m, $CH_3OOCCH_2CH_2$-17), 2.01 (3H, t, J = 7.6 Hz, CH_3CH_2-8), 1.89 (3H, d, J = 7.1 Hz, CH_3-18), 1.71 (3H, t, J = 7.8 Hz, CH_3CH_2-3), −0.59 (1H, br, −NH), 0.64 (1H, br, −NH). MS (FAB) m/z 1249.8 (MH^+, 100). HRMS (FAB): calcd for $C_{72}H_{81}N_8O_{12}$ 1249.5973; found 1249.6000. Spectral data for **24**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 409 (225524), 499 (24913), 603 (10927), 655 (67963). ^{1}H NMR data (see Table 2). MS (FAB) m/z 1249.8 (MH^+, 100). HRMS (FAB): calcd for $C_{72}H_{81}N_8O_{12}$ 1249.5973; found 1249.5980.

Thermolysis of *vic*-7,8-Dihydroxymesopurpurin-18-*N*-hexylimide Methyl Ester 19 (25−27). Diol **19** (519 mg) was treated with *o*-dichlorobenzene (50 mL) as described in the general procedure. The purification was carried out with silica gel column by eluting with dichloromethane/ethyl acetate (v/v 20/1) to give the 8-vinylchlorin **25** (201 mg, 41%), then eluting with 10% MeOH/CH_2Cl_2 to give a mixture. The mixture was further purified by preparative silica gel TLC plates, developed with hexanes/dichloromethane/ethyl acetate (v/v/v 4/1/2). Two major bands were collected from the plates. The first band (faster moving) was identified as 8-ketobacteriochlorin **26** (39 mg, 8%). The second band was identified as unsymmetrical dimer **27** (61 mg, 13%). Spectral data for **26**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 411 (107588), 479 (5117), 511 (8887), 548 (21814), 668 (11850), 725 (34471). ^{1}H NMR data (see Table 1). MS (FAB) m/z 679.5 (M^+, 100). HRMS (FAB): calcd for $C_{40}H_{50}N_5O_5$ 680.3812; found 680.3807. Spectral data for **27**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 414 (215000), 510 (19000), 546 (21000), 636 (16500), 699 (87000). ^{1}H NMR data (see Table 2). MS (FAB) m/z 1323.2 (MH^+, 100). HRMS (FAB): calcd for $C_{80}H_{94}N_{10}O_8$ 1322.7243; found 1332.7255.

Thermolysis of *vic*-7,8-Dihydroxymesopurpurin-18-*N*-3,5-bis(trifluoromethyl)benzylimide Methyl Ester 20 (28−30). Diol **20** (243 mg) was treated with *o*-dichlorobenzene (30 mL) as described in the general procedure. The purification was carried out with a silica gel column by eluting with dichloromethane/acetone (v/v 99/1) to give the 8-vinylchlorin **28** (120 mg, 51%), then eluting with 10% MeOH/CH_2Cl_2 to give a mixture. The mixture was further purified by preparative silica gel TLC plates, developed with hexanes/acetone (v/v 7/3). Two major bands were collected from the plates. The faster moving band was identified as 8-ketobacteriochlorin **29** (15 mg, 6%). The second band was identified as unsymmetrical dimer **30** (36 mg, 15%). Spectral data for **29**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 411 (121904), 479 (4963), 512 (9306), 549 (26831), 668 (13338), 726 (36292). ^{1}H NMR data (see Table 1). MS (FAB) m/z 821.4 (M^+, 100). HRMS (FAB): calcd for $C_{43}H_{41}F_6N_5O_5$ 821.3012; found 821.3013. Spectral data for **30**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 366 (88568), 426 (234354), 512 (21609), 547 (36523), 638 (17044), 693 (82176). ^{1}H NMR data (see Table 2). MS (FAB) m/z 1607.6 (MH^+, 100). HRMS (FAB): calcd for $C_{86}H_{79}F_{12}N_{10}O_8$ 1607.5890; found 1607.5870.

The Preparation of Porphyrin−Chlorin Dimer 39. A solution of *vic*-7,8-dihydroxychlorins **19** (60 mg) and rhodoporphyrin IX dimethyl ester **38** (60 mg) in *o*-dichlorobenzene (30 mL) was stirred in an atmosphere of nitrogen at 150 °C for 4 h. After being cooled to room temperature, the reaction mixture was loaded on a short silica column, eluted with hexanes to remove *o*-dichlorobenzene, then eluted with 10% MeOH/CH_2Cl_2 to give a mixture. The mixture was purified by preparative silica plates, developed with dichloromethane/acetone (v/v 40/1). Porphyrin−chlorin dimer **39** (16 mg, 30%) was obtained. UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 414 (165000), 420 (180000), 510 (15000), 546 (33000), 588 (12000), 636 (15000), 699 (44000). ^{1}H NMR (600 MHz, $CDCl_3$): 10.98 (1H, s, H-15), 10.03 (1H, s, H-10), 9.89 (1H, s, H-20), 9.77 (2H, s, H-5 and H-10′), 9.69 (1H, s, H-5′), 8.51 (1H, s, H-20′), 8.12 (1H, d, J = 16.0 Hz, H-3^1), 7.35 (1H, dt, J = 16.0, 5.7 Hz, H-3^2), 5.43 (1H, dd, J = 8.9, 2.9 Hz, H-17′), 5.06 (2H, br d, J = 5.6 Hz, H-7′^{1}), 4.49 (2H, m, $CH_3OOCH_2CH_2$-17), 4.40 (3H, s, CH_3OOC-13), 4.38 (3H, m, H-18′ and $-NCH_2CH_2CH_2CH_2CH_2CH_3$), 3.97 (2H, q, J = 7.7 Hz, CH_3CH_2-8′), 3.89 (2H, q, J = 7.7 Hz, CH_3CH_2-8), 3.88 (3H, s, CH_3-12′), 3.87 (3H, s, CH_3-12), 3.68 (2H, q, J = 7.9 Hz, CH_3CH_2-3′), 3.65 (3H, s, $-COOCH_3$), 3.58 (3H, s, CH_3-18), 3.57 (3H, s, $-COOCH_3$), 3.54 (3H, s, CH_3-2), 3.29 (2H, t, J = 7.8 Hz, $CH_3OOCH_2CH_2$-17), 3.15 (3H, s, CH_3-2′), 2.81 (3H, s, CH_3-7), 2.71 and 2.40 and 2.02 (1H, 2H, 3H, m, CH_3-

TABLE 2. ^{1}H NMR Spectral Data of Chlorin−Chlorin Dimers 8, 11, 24, 27, and 30[a]

	8	**11**	**24**	**27**	**30**
2-CH_3	3.30, s	3.24, s	3.26, s	3.20, s	3.19, s
3-CH_2CH_3	3.81, m	3.73, q, J = 7.7	3.77, q, J = 7.6	3.68, q, J = 7.8	3.68,[c] m
3-CH_2CH_3	1.70, t, J = 7.7	1.67, t, J = 7.3	1.68, t, J = 7.5	1.65, t, J = 7.6	1.65, t, J = 7.6
5-H	9.33, s	9.12, s	9.27, s	9.13, s	9.11, s
7-CH_3	3.32, s	3.26, s	3.27, s	3.17, s	3.16, s
8^1	8.02, d, J = 15.9	7.84, d, J = 15.9	7.95, d, J = 15.6	7.79, d, J = 15.8	7.76, d, J = 16.2
8^2	7.25, dt, J = 15.8, 6.0	7.24, dt, J = 16.0, 6.0	7.20, dt, J = 15.9, 6.0	7.14, dt, J = 15.9, 5.9	7.12, dt, J = 15.9, 5.8
10-H	9.59, s	9.27, s	9.61, s	9.52, s	9.46, s
12-CH_3	2.68, s	2.84, s	2.81, s	3.18, s	3.09, s
15-CH_2CO−	5.17, AB system, J = 18.4	5.06, AB system, J = 20.0			
17-H	4.35, m	4.20, m	5.08, m	5.35, dd, J = 9.0, 2.6	5.27, dd, J = 9.1, 1.7
17-$CH_2CH_2CO_2CH_3$ and 17′-$CH_2CH_2CO_2CH_3$	2.54 (2H, m), 2.18 (4H, m), 1.75 (2H, m)	2.80−2.14, m	2.55−1.96, m	2.76−2.62 (2H, m), 2.53−2.27 (4H, m), 2.10−1.91 (2H, m)	2.70 (2H, m), 2.39 (4H, m), 1.95 (2H, m)
18-H	4.35, m	4.41, m	4.33, q, J = 7.3	4.30, q, J = 7.4	4.29, q, J = 7.3
18-CH_3	1.69, d, J = 8.0	1.77, d, J = 7.1	1.80, d, J = 7.3	1.72, d, J = 7.4	1.72, d, J = 7.3
20-H	8.60, s	8.42, s	8.55, s	8.47, s	8.44, s
2′-CH_3	3.28, s	3.24, s	3.24, s	3.20, s	3.17, s
3′-CH_2CH_3	3.81, m	3.77, q, J = 7.7	3.77, q, J = 7.6	3.72, q,J = 7.8	3.68,[c] m
3′-CH_2CH_3	1.57, t, J = 7.5	1.55, t, J = 7.7	1.56, t, J = 7.5	1.54, t, J = 7.7	1.52, t, J = 7.6
5′-H	9.82, s	9.64, s	9.75, s	9.61, s	9.56, s
7′^{1}	5.13, d, J = 6.7	5.01,[b] d	5.08, m	4.95, br d, J = 5.7	4.94, br d, J = 5.7
8′-CH_2CH_3	4.07, m	3.92, q, J = 7.6	4.02, q, J = 7.6	3.90, q, J = 7.8	3.89, q, J = 7.7
8′-CH_2CH_3	1.95, t, J = 7.4	1.92, t, J = 7.8	1.93, t, J = 7.8	1.90, t, J = 7.7	1.88, t, J = 7.7
10′-H	9.84, s	9.57, s	9.87, s	9.73, s	9.71, s
12′-CH_3	3.63, s	3.66, s	3.70, s	3.86, s	3.84, s
15′-CH_2CO−	5.31, AB system, J = 18.9	5.22, dd, J = 20.0			
17′-H	4.42, m	4.31, m	5.17, dd, J = 9.2, 2.8	5.44, dd, J = 9.0, 2.7	5.36, dd, J = 9.1, 2.0
18′-H	4.42, m	4.50, m	4.39, q, J = 7.4	4.38,[c] m	4.36, q, J = 7.3
18′-CH_3	1.77, d, J = 6.4	1.86, d, J = 7.1	1.87, d, J = 7.1	1.80, d, J = 7.4	1.80, d, J = 7.2
20′-H	8.65, s	8.51, s	8.61, s	8.54, s	8.51, s
$-CO_2CH_3$	3.60, 3.64, 3.70, 3.78, 4.08, 4.28, all 3H, all s	3.61, 3.67, both 3H, both s	3.50, 3.54, 4.07, 4.08, 4.19, 4.25, all 3H, all s	3.55, 3.59, both 3H, both s	3.54, 3.57, both 3H, both s
4 × −NH	−1.10, −1.15, −1.36, −1.46, each br s	0.72, 0.34, −1.44, −1.74, each br s	−0.64, −0.68, −0.88, −0.98, each br s	0.15, −0.01, −0.09, −0.23, each br s	0.39, 0.19, 0.15, −0.02, each br s
2 × $-NCH_2C_6H_3(CF_3)_2$[d]	8.27 (2H, s), 8.17 (2H, s), 7.83 (1H, s), 7.80 (1H, s), 5.78 (2H, s), 5.68 (2H, s)				
2 × $-NCH_2(CH_2)_4CH_3$[e]	4.49 (2H, m), 4.38 (2H, m),[c] 2.10−1.91 (4H, m), 1.59 (4H, m), 1.52−1.37 (8H, m), 0.97 (3H, t, J = 7.4), 0.95 (3H, t, J = 7.2)				

[a] All NMR spectra were recorded at 400 MHz. The coupling constant values *(J)* are expressed in hertz. [b] Overlapped with 15-CH_2CO−. [c] Overlapped with each other. [d] For compound **30** only. [e] For compound **27** only.

TABLE 3. Fluorescence Spectral Data of Dimer 7 and the Corresponding Mono- and Di-Zn(II) Complexes 31 and 32, Respectively, at Equimolar Concentration (1.0 mM) in Dichloroomethane

dimers	excitation wavelength (nm)	emission wavelength (nm)
7	416	659
	653	659
31	417	658
	628	659
	653	658
32	411	636
	627	634

OOCH_2CH_2-17′ and $-NCH_2CH_2CH_2CH_2CH_2CH_3$), 1.96 (3H, t, J = 7.6 Hz, CH_3CH_2-8′), 1.78 (3H, d, J = 7.4 Hz, CH_3-18′), 1.69 (3H, t, J = 7.7 Hz, CH_3CH_2-8), 1.63 (2H, m, $-NCH_2CH_2CH_2CH_2CH_2CH_3$), 1.47 (3H, t, J = 7.7 Hz, CH_3CH_2-3′), 1.44 (4H, m, $-NCH_2CH_2CH_2CH_2CH_2CH_3$), 0.97 (3H, t, J = 7.3 Hz, $-NCH_2CH_2CH_2CH_2CH_2CH_3$), 0.20 (1H, br s, −NH), 0.07 (2H, br s, 2 × −NH), −3.69 (1H, br s, −NH). MS (FAB) *m*/*z* 1225.6 (M^+, 100). HRMS (FAB): calcd for $C_{74}H_{83}N_9O_8$ 1225.6364; found 1225.6353.

The Preparation of Mono-Zn(II) Dimer 31 and Di-Zn-(II) Dimer 32. A solution of zinc acetate dihydrate (26 mg) in methanol (10 mL) was added to a solution of dimer **7** (38 mg) in dichloromethane (12 mL). The mixture was stirred at room temperature for 1 h. After workup and removal of solvent, the residue was subjected on preparative silica plates, developed with dichloromethane/ethyl acetate (v/v 5/1). The starting material **7** (14 mg) was recovered. Mono-Zn dimer **31** (15 mg, 60% converted yield) and di-Zn dimer **32** (4 mg, 15% converted yield) were obtained. The di-Zn dimer **32** (25 mg, 95%) was also prepared by treatment of dimer **7** (24 mg) with a large excess of zinc acetate dihydrate (1.0 g). Spectral data for **31**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 417 (260939), 503 (47736), 594 (32718), 628 (70531), 653 (56854). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.89 (1H, s, H-10), 9.84 (2H, s, H-5 and H-5′), 9.72 (1H, s, H-10′), 8.77 (2H, s, H-7^{1}and H-7′^{1}), 8.69 (1H, s, H-20), 8.51 (1H, s, H-20′), 5.35−5.03 (4H, m, AB system, CH_3COOCH_2-15 and CH_3COOCH_2-15′), 4.34 (4H, m, H-17, H-18 and H-17′, H-18′), 4.30 (2H, m, CH_3CH_2-8), 4.24 (2H, m, CH_3CH_2-8′), 4.23 (6H, s, 2 × CH_3COO−), 3.86 (3H, s, CH_3COO−), 3.80 (2H, m, overlapped, CH_3CH_2-3), 3.79 (3H, s, CH_3COO−), 3.75 (2H, m, overlapped, CH_3CH_2-3′), 3.64 (3H, s, CH_3COO−), 3.61 (3H, s, CH_3COO−), 3.60 (3H, s, CH_3-12), 3.52 (3H, s, CH_3-12′), 3.34 (3H, s, CH_3-2), 3.23 (3H, s, CH_3-2′), 2.56,

2,22, 1.80 (2H, 4H, 2H, m, $CH_3COOCH_2CH_2$-17 and $CH_3COOCH_2CH_2$-17′), 2.12 (3H, t, J = 7.5 Hz, CH_3CH_2-8), 2.09 (3H, t, J = 7.6 Hz, CH_3CH_2-8′), 1.79 (6H, d, J = 7.4 Hz, CH_3-18 and CH_3-18′), 1.70 (6H, two triplets, overlapped, J = 7.0 Hz, CH_3CH_2-3 and CH_3CH_2-3′), −1.07 (1H, br, −*NH*), −1.12 (1H, br, −*NH*). MS (FAB) *m*/*z* 1339.6 (MH^+, 100). HRMS (FAB): calcd for $C_{74}H_{83}N_8O_{12}Zn$ 1339.5421; found 1339.5460. Spectral data for dimer **32**, UV−vis in CH_2Cl_2 [λ_{max} (ϵ)]: 411 (135471), 585 (25883), 627 (65808). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.78 (1H, s, H-5 or H-10), 9.71 (1H, s, H-5 or H-10), 8.76 (1H, s, H-7^1), 8.49 (1H, s, H-20), 5.13 (2H, AB system, J = 19.6 Hz, CH_3COOCH_2-15), 4.42−4.22 (4H, m, H-17, H-18 and CH_3CH_2-8), 4.20 (3H, s, CH_3COO−), 3.84 (3H, s, CH_3COO−), 3.74 (2H, q, J = 7.1 Hz, CH_3CH_2-3), 3.61 (3H, s, CH_3COO−), 3.49 (3H, s, CH_3-12), 3.22 (3H, s, CH_3-2), 2.56, 2,22, 1.82 (1H, 2H, 1H, m, $CH_3COOCH_2CH_2$-17), 2.10 (3H, t, J = 7.5 Hz, CH_3CH_2-8), 1.77 (3H, d, J = 7.0 Hz, CH_3-18), 1.70 (3H, t, J = 7.5 Hz, CH_3CH_2-3). MS (FAB) *m*/*z* 1404.5 (M^+, 100). HRMS (FAB): calcd for $C_{74}H_{80}N_8O_{12}Zn_2$ 1400.4480; found 1400.4510.

Acknowledgment. The authors are grateful to the NIH (CA 55791) for the financial support. Partial support by shared resources of the Roswell Park Cancer Support Grant (P30CA16056) is also acknowledged. We are also thankful to Dr. Alderfer and other members of the NMR Facility for their help. The M.S. analyses were obtained by Berverly Chamberlin, Mass Spectrometry Facility, Michigan State University, East Lansing.

JO0207630