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Syntheses of Stable Bacteriochlorophyll-a Derivatives As Potential Photosensitizers For Photodynamic Therapy

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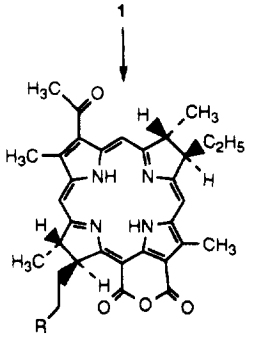
Abstract: *New methods for conversion of unstable bacteriochlorophyll-a present in Rb. sphaeroides into stable bacteriochlorins are presented. Cyclic imide derivatives from related cyclic isoimide or amide analogs are obtained by intramolecular base catalyzed cyclization. Most of the new bacteriochlorins have long wavelength absorptions in the range of 796-822 nm. In preliminary screening, the isoimide analogs have shown promising in vivo photosensitizing activity for the treament of cancer by photodynamic therapy.*

Photofrin®, a hematoporphyrin derivative, has now been approved in the United States and worldwide for the treatment of various types of cancers by photodynamic therapy (PDT).[1] Porphyrins in general, including Photofrin®, have weak absorption in the red region (up to 630 nm) of the optical spectrum. Thus, efforts are currently being made by various research groups to develop photosensitizers which are chemically pure and have absorption near 800 nm[2]. Due to the deeper tissue penetration of the activating light, and also the availability of less expensive diode lasers in this region of the spectrum, such photosensitizers should make PDT more efficient and practical. Naturally occurring bacteriochlorins have been reported[3] to show *in vivo* sensitizing efficacy, but were found to be quite unstable under PDT protocols. Thus, the syntheses of stable bacteriochlorins[4] with appropriate photochemical properties has been an elusive objective of our laboratories.

Rb. sphaeroides
(contains bacteriochlorophyll-a)

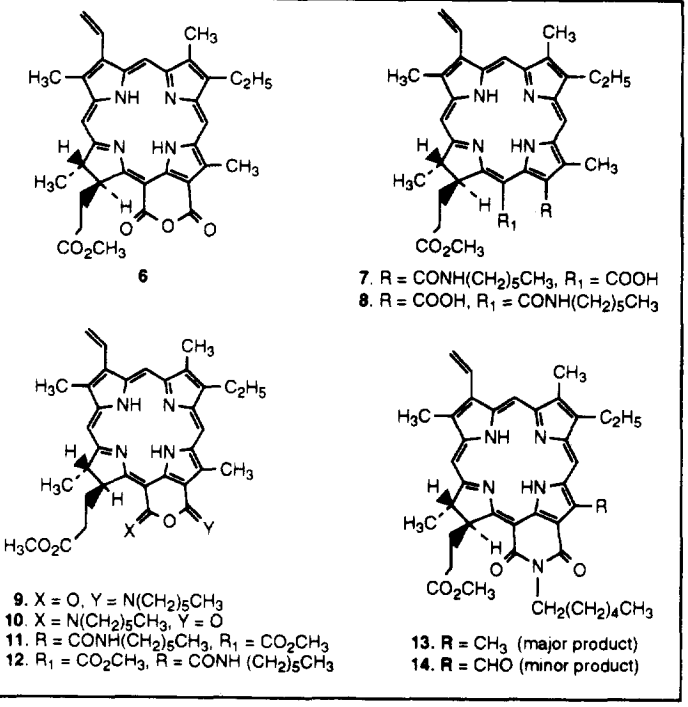


2. R = $CO_2(CH_2)_2CH_3$ **3**. R = COOH
4. R = $CONHCH(CO_2Bu^t)CH_2(CO_2Bu^t)$
5. R = $CONHCH(CO_2Bz)CH_2CO_2Bz$

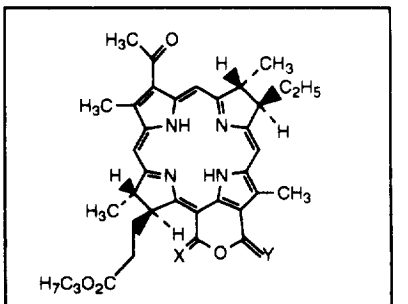
In our previous studies with several chlorins we observed that introduction of a cyclic anhydride ring generally enhanced the stability of the compound towards oxidation.[5] These results prompted us to investigate the effect of such a cyclic anhydride and also more stable imide rings in naturally occurring bacteriochlorins. The reaction conditions previously reported by us for isolation of bacteriopurpurin **3** were optimized.[6] Thus, the n-propyl alcohol extract of *Rb.sphaeroides*,[7] which contains bacteriochlorophyll-a **1** (λ max 774 nm), was directly reacted with KOH/n-propanol. Air was continuously bubbled through the reaction mixture. The intermediate "unstable bacteriochlorin" was not isolated and was immediately treated with 1N HCl to produce bacteriopurpurin-a propyl ester **2** and the related carboxylic acid **3** in 30-35% yield. Compared with bacteriochlorophyll-a **1**, bacteriopurpurins **2** and **3** were found to be *extremely stable* at room temperature, with strong wavelength

absorption at 813 nm. These bacteriochlorins had the "ideal" photochemical properties required for an effective PDT agent. However, due to their low solubility in most of the injectable solvents, these compounds could not be evaluated for *in vivo* photosensitizing efficacy. Conversion of **3** into the corresponding aspartic acid di-*tert*- butyl esters **4** and aspartic acid di-benzyl esters **5** slightly improved their solubility. In our quest to compare the effect of cyclic anhydride vs imide analogs for quantitative structure activity relationship (QSAR) studies, several unsuccessful attempts were made to convert the cyclic anhydride **2** into the more stable imide analog **13** by following literature procedures[8] common in other aromatic systems.

Model studies were then performed by using a less expensive substrate such as purpurin-18 methyl ester **6**. As expected[5], reaction of **6** (λ max 700 nm) with 1-hexylamine at room temperature gave the corresponding amides, in 95% yield, as a mixture of **7** and **8** in the ratio of 6 to 1(determined using ^{1}H NMR) and with λ max at 665 nm. In order to obtain the desired cyclic imides, we thought it necessary to activate the carboxylic function of the intermediate amides, which on base treatment could generate the desired product by intramolecular cyclization. For our studies, two methods were investigated. In the first approach, the carboxylic acids, (mixture of **7** and **8**) were reacted with DCC[9] to produce cyclic isomides **9** (690 nm) and **10** (696 nm), in a ratio of 6:1, in 96% overall yield. Under basic reaction conditions, treatment of isoimides with DBU/toluene at 60°C produced purpurin-imide **13** in low yield. Interestingly, replacing DBU with stronger bases, such as, methanolic KOH or NaOH) at room temperature, gave the desired purpurin-imide **13** in 85% yield as a major product (λ max 705nm). In our second approach, the intermediate amide mixture **7** and **8** was converted into the corresponding methyl esters **11**, **12** by reacting with diazomethane; upon brief methanolic KOH treatment the desired imide analog was obtained. Under these conditions, cyclic imide formation at the last step of intramolecular cyclization produced the desired imide **13** in excellent yield (>80%). However, a small amount of slow moving green chromatographic band was also isolated. This minor product was identified as the 12-formylpurpurin imide **14** (λ max: 714 nm). To our knowledge, this is the first example of the formation of 12-formylchlorin from related amide analogs. Currently, the mechanism of the formation of the formyl derivative **14** is being investigated by using individual isomers **9-12** as substrates under various reaction conditions.

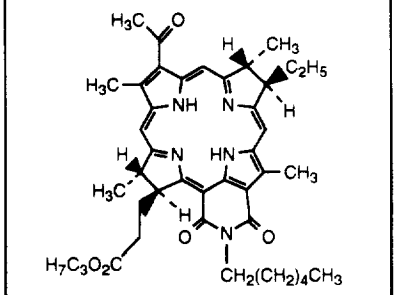


The optimized conditions established in our model studies were then employed for the preparations of the related bacteriochlorin derivatives. Reaction of amide analogs, obtained as a mixture by cleaving the cyclic anhydride ring in **2** with 1-hexylamine was converted into unstable carbodiimide analogs[9] which rapidly converted into more stable cyclic isoimides and were separated into individual isomers **15** (804 nm) and **16** (796 nm) in a 6:1 ratio by preparative chromatography. Intramolecular base catalyzed cyclization of the isoimides, either as a mixture or as pure isomers, generated the desired imide **19** (λ max 822 nm) in 45 % yield. Similar results were obtained from amides **17** and **18**, in which the carboxylic acid functionalities were converted into the related methyl esters before base treatment. Surprisingly, unlike natural bacteriochlorophyll-a, the corresponding isoimide analogs **15** and **16**, as well as imide derivative **19**, were found to be extremely stable. Besides their remarkable stability, conversion of the cyclic anhydride into corresponding imides and isoimides also produced significant increase in solubility in various injectable solvents.



15. X = O, Y = $N(CH_2)_5CH_3$
16. X = $N(CH_2)_5CH_3$, Y = O

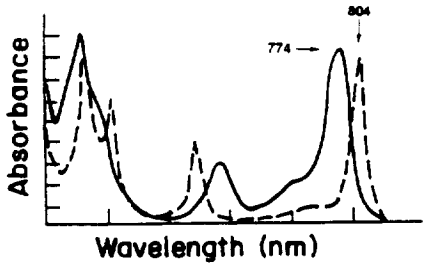
17. R_{13} = $CONH(CH_2)_5CH_3$, R_{14} = CO_2CH_3
18. R_{13} = CO_2CH_3, R_{14} = $CONH(CH_2)_5CH_3$



19

Among all the bacteriochlorins synthesized so far, only the isoimide analogs **15** and **16** (as a mixture) have been evaluated for PDT efficacy in DBA/2 mice transplanted with RIFT and SMT-F tumors. In a preliminary screening, these compounds showed promising photosensitizing activity at a very low dose (0.25 μmol/kg) in both tumor models. The mice were treated with laser light (804 nm, 75mW/cm^2, 135 J/cm^2) at 24h post injection of the drug. The biological studies with pure isoimides and related imide analogs bearing various N-substituents, at different doses and time intervals, are currently in progress and will be reported elsewhere.

In summary, we have shown that naturally occurring unstable bacteriochlorophyll-a, can be converted into stable bacteriochlorins with *remarkable stability* and promising *in vivo* photosensitizing efficacy. For QSAR studies, syntheses of a series of N-substituted cyclic imides and isoimides with known target specific binding sites are currently in progress. Detailed biological studies with these compounds might help us to understand the binding sites of various photosensitizers and their mode of action, which are still not known.



Optical spectra (in CH_2Cl_2) of bacteriochlorin **15** (804 nm) and bacteriochlorophyll-a **1** (774 nm)

All new compounds were characterized by NMR and mass spectrometry.[10]

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10. ^{1}H NMR (400 MHz, expressed in δ ppm) and Mass Spectrometry Data:

12-Formyl-N-hexylimide-17-purpurin-18 methyl ester (**14**): 11.87 (s, 1H, 12-CHO), 10.21 (s, 1H, 5- H), 8.79 (s, 1H, 10-H), 8.32 (s, 1 H, 20-H), 7.68 (dd, 1H, *J* =18.8, *J* =12.5, 3a-H), 6.24 (d, 1H,*J* =18.8, 3b-H), 6.15 (d, 1H, *J* =12.5, 3b'-H), 5.23 (d, 1H, *J* =8.6, 17-H), 5.47 (t, 2H, *J* =7.8, hexylimide-*a*-CH_2), 4.19 (q, H, *J* =8.0, 18-H), 3.69 (s, 3H, CO_2Me), 3.47(q, 2H, *J* = 7.6, 8a-H), 3.25 (s, 3H, 7-Me), 2.97 (s, 3H, 2-Me), 2.73 (m, H, 17b-H), 2.41 (m, 2H, 17a'-H and 17b'-H), 1.98 (m, 5H, 17a-H and *b,c*-N-hexyl-CH_2), 1.72 (d, 3H, *J* = 8.0, 18-Me), 1.41 (t, 3H, *J* =7.6, 8b-Me), 1.32 (m,4H, *d,e*-hexylimide-CH_2), 0.83 (t, 3H, 18-Me), -0.13 and -0.46 (each br s, 2H, 21 and 23-NH). Mass calculated for $C_{40}H_{45}N_5O_5$: 675. Found: 676 (M+1).

13^1-N-Hexylisoimide-bacteriopurpurin-a propyl ester (**15**): 9.38 (s, 1H, 5- H), 8.88 (s, 1H, 10-H), 8.73 (s, 1 H, 20-H), 5.46 (m, 1H, NHCO), 5.18 (d, 1H, *J* =8.0, 17-H), 4.34 (m, 2H, 7-H and 18-H), 4.17 (m, 1H, 8-H), 3.91 (m, 2H, hexylisoimide-*a*-CH_2), 4.06 (t, 2H, CO_2CH_2), 3.68 (s, 3H, 12-Me), 3.59 (s, 3H, 2-Me), 3.19 (s, 3H, 3-Me), 2.73 (m, H, 17b-H), 2.42 (m, 5H, $CH_2CH_2CH_3$ and 8a-CH_2 and 7b'-H), 2.14 (m, H, 17a-H), 2.08 (m, 5H, hexylisoimide-*b,c*- CH_2 and 17a'-H), 2.01, 1.93 (each d, 3H, *J* =8.0 and 18-Me, 7-Me), 1.57 (m, 4H, hexylisoimide-*d,e* -CH_2), 1.12 (t, 3H, *J* =7.8, 3b-Me), 0.96 (t, 3H, hexylisoimide-*f*- CH_3), 0.87 (t, 3H, *J* =8.2, $CH_2CH_2CH_3$), -0.86 and -1.13 (each br s, 2H, 21 and 23-NH). Mass calculated for $C_{42}H_{55}N_5O_5$: 707. Found: 708 (M+1).

13^3-N-Hexylisoimide-bacteriopurpurin-a propyl ester (**16**): 9.21 (s, 1H, 5- H), 8.77 (s, 1H, 10-H), 8.68 (s, 1 H, 20-H), 5.35 (m, 2H, NHCO + 17-H), 4.32 (m, 2H, 7-H and 18-H), 4.11 (m, 3H, 8-H and hexylisoimide-*a*-CH_2), 3.91 (t, 2H, CO_2CH_2), 3.69 (s, 3H, 12-Me), 3.58 (s, 3H, 2-Me), 3.19 (s, 3H, 3-Me), 2.62 (m, H, 17b-H), 2.44 (m, 5H, CH_2CH_2 CH_3 and 8a-CH_2 and 7b'-H), 2.14 (m, 6H, 17a-H and hexylisoimide-*b,c*- CH_2+17a'-H), 1.93, 1.84 (each d, 3H,*J* =8.0, 18-Me and 7-Me), 1.60 (m, 4H, hexylisoimide-*d,e*--CH_2), 1.11 (t, 3H, *J* =7.8, 3b-Me), 0.97 (t, 3H, hexylisoimide-*f*-CH_3), 0.88 (t, 3H,*J* =8.2, $CH_2CH_2CH_3$), -0.68 and -1.03 (each br s, 2H, 21 and 23-NH). Mass calculated for $C_{42}H_{55}N_5O_5$: 707. Found: = 708 (M+1).

N-Hexylimide-bacteriopurpurin-a propyl ester (**19**): 9.31 (s, 1H, 5- H), 8.80 (s, 1H, 10-H), 8.63 (s, 1 H, 20-H), 5.29 (d, 1H, *J* =8.7, 17-H), 4.42 (t, 2H, *J* =7.8, hexylimide-*a*-CH_2), 4.29 (m, H, 3-H), 4.09 (m, 3H, CO_2CH_2 and 18-H), 3.94 (m, 2H, 7-H and 8-H), 3.70 (s, 3H, 12-Me), 3.55 (s, 3H, 2-Me), 3.17 (s, 3H, 3-Me), 2.68 (m, 1H, 17b-H), 2.41 (m, 5H, $CH_2CH_2CH_3$ + 8a-CH_2 + 7b'-H), 2.04 (m, 2H, 17a-H, 17a'-H and *b,c*-N-hexyl-CH_2), 1.70, 1.67 (each d, 3H, *J* =8.0, 18-Me, 7-Me), 1.32 (m, 4H, *d,e*--hexylimide-CH_2), 1.14 (t, 3H, *J* =7.8, 3-b Me), 0.93 (t, 3H, *J* =8.2, $CH_2CH_2CH_3$), -0.53 and -0.75 (each br s, 2H, 21and 23-NH). Mass calculated for $C_{42}H_{55}N_5O_5$: 707 Found: 707.9 (M+1).

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