Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 10, pp. 1263-1267, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0139-6

SYNTHESES OF NEW BACTERIOCHLORINS AND THEIR ANTITUMOR ACTIVITY

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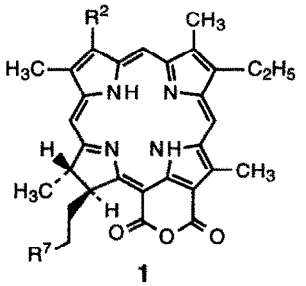
Abstract: Purpurin-18 methyl ester **1a** was converted into a series of new bacteriochlorins **2b, 2c, 2f** and **4b**. These stable bacteriochlorins have strong long wavelength absorptions in the range of 760 to 816 nm, and were tested for *in vivo* photosensitizing activity using the standard screening system of DBA/2 mice bearing transplanted SMT/F tumors. In preliminary screening, among the photosensitizers tested so far, bacteriochlorins **2f** and **4b** have shown promising anti-tumor activity.

Among long wavelength absorbing photosensitizers, bacteriochlorins have been proposed as potentially useful candidates for use in photodynamic therapy (PDT) where strong absorptions in the visible spectrum can be used to photoactivate dyes previously located in targeted (neoplastic) tissues.[1] Some naturally occurring bacteriochlorins have previously been reported as effective photosensitizers both *in vitro* and *in vivo*.[2] However, most of the naturally occurring bacteriochlorins (760-780 nm) are extremely sensitive to oxidation, which results in rapid transformation to the chlorin state (ca. 640 nm). Furthermore, if a laser is used to excite the bacteriochlorin *in vivo*, oxidation may result in the formation of a new chromophore absorbing outside the laser window, thus reducing the photodynamic efficiency. In order to render PDT more generally applicable to tumor therapy there is a need for long wavelength absorbing photosensitizers, such as stable bacteriochlorins, which have the ability to localize in high concentration at the tumor site.

Chang et al.[3] have previously shown that chlorins react with osmium tetraoxide to give the *vic* dihydroxy-bacteriochlorin system. We extended this methodology to the pheophorbide-a and chlorin e_6 series, and prepared a series of *vic* -dihydroxy- and keto-bacteriochlorins.[4] We also reported that the regiospecificity of pyrrole subunit modification in the osmium tetraoxide oxidation is affected significantly by the presence of electron-withdrawing substituents on the macrocycle.[4] Though the stable bacteriochlorins prepared from mesochlorin-e_6 trimethyl ester and methyl pyropheophorbide-a had strong absorption in the red region of their electronic spectra (730 to 750 nm) they did not show any significant *in vivo* photosensitizing activity.[5]

A few years ago, Hoober et al.[6] showed that purpurin-18 methyl ester, **1a**, which has a strong absorption maximum at 700 nm, might be a useful photosensitizer for PDT. In order to prepare long wavelength absorbing photosensitizers, we modified purpurin-18, and two new bacteriochlorin systems **2**, (in which a six membered anhydride ring is fused to the macrocycle) and **4**, (in which the anhydride ring is replaced by imide ring system)[7] were synthesized. Purpurin-18 methyl ester **1a** was obtained from methyl pheophorbide-a by following the literature procedure.[8] The anhydride ring in **1a** was replaced with imide ring system **3** by first reacting purpurin-18 with lysine at room temperature. The intermediate, which has strong absorption at 660 nm was isolated in 80% yield. Various reaction

conditions[7] were used to convert the open chain amide into its cyclic derivative **3a**, and the best results were obtained when the intermediate amide was stirred with Montmorillonite K10 clay (Aldrich) in dichloromethane. The reaction was monitored using spectrophotometry. Like purpurin-18 methyl ester **1a**, the cyclic imide **3a** also had a strong absorbance maximum at 702 nm. The other imide derivative **3c**, was prepared by following a similar approach, except purpurin-18 methyl ester **1** was reacted with 1-hexylamine instead of L-lysine. For the preparation of formyl analogues **1b**, **3b** and **3d**, the corresponding vinyl derivatives **1a**, **3a** and **3c** were reacted with osmium tetraoxide/sodium periodate,[9] and were isolated in excellent yield.



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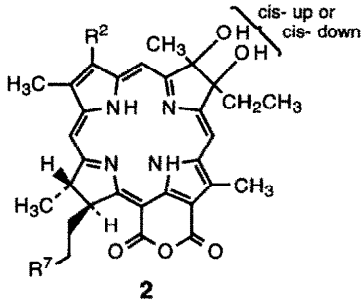


2

a. $R^2 = CH{=}CH_2$; $R^7 = CO_2CH_3$ **d.** $R^2 = CH{=}CH_2$; $R^7 = CO_2H$

b. $R^2 = CHO$; $R^7 = CO_2CH_3$ **e.** $R^2 = CH{=}CH_2$; $R^7 = CONHCH(CO_2Bu^t)CH_2CO_2Bu^t$

c. $R^2 = CH_2CH_3$; $R^7 = CO_2CH_3$ **f.** $R^2 = CHO$; $R^7 = CONHCH(CO_2Bu^t)CH_2CO_2Bu^t$

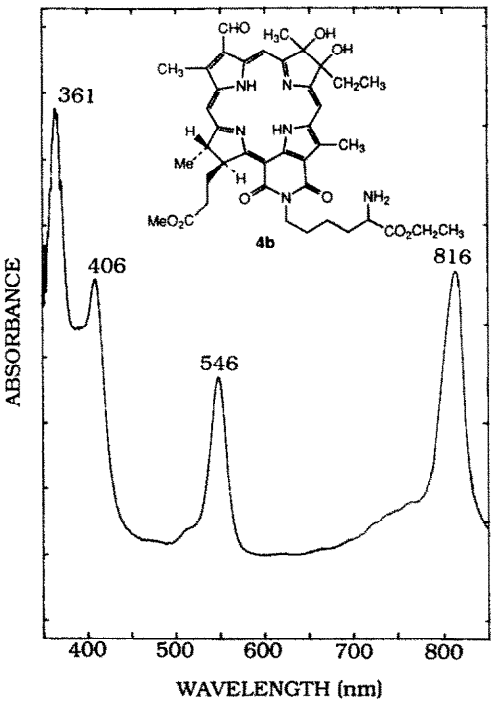
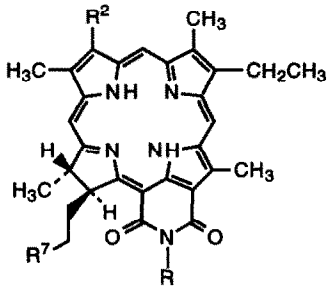


Figure 1: Optical spectrum, in CH_2Cl_2, of lysyl imide **4b**.

We and others[10] have shown that the PDT effectiveness of certain photosensitizers can be improved by converting the methyl esters into the corresponding aspartyl derivatives. In order to probe the effect of such substituents in our newly synthesized bacteriochlorin systems, purpurin-18 methyl ester **1** was converted into the corresponding carboxylic acid by acidic hydrolysis. It was then reacted with DCC, aspartic acid di-tert-butyl ester and a catalytic amount of N,N-dimethylaminopyridine in tetrahydrofuran. The desired aspartic derivative was isolated in 75% yield. Other derivatives were prepared in almost the same yield by following the same approach. The formyl derivatives, e.g. **3i**, were prepared by following the methodology as discussed for the methyl ester analogues. For the synthesis of *vic*- dihydroxy-bacteriochlorins **2c**, **2f** and **4b**, the respective vinyl analogues were individually reacted with osmium tetraoxide/pyridine, and then with hydrogen sulfide gas.[3,4] After column chromatographic purification on silica gel the desired products were isolated in 60-70% yields. Figure 1 shows the optical spectrum of the imide **4b** (λ_{max} 816 nm); the optical

spectrum of the corresponding purpurin-18 bacteriochlorin analogue was virtually superimposable, except that its long-wavelength maximum appeared at 813 nm.



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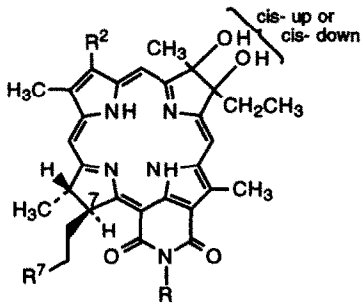


4

a. $R^2 = CH{=}CH_2$; $R^7 = CO_2CH_3$
$R = (CH_2)_4CH(CO_2Et)NH_2$

b. $R^2 = CHO$; $R^7 = CO_2CH_3$
$R = (CH_2)_4CH(CO_2Et)NH_2$

c. $R^2 = CH{=}CH_2$; $R^7 = CO_2CH_3$
$R = (CH_2)_5CH_3$

d. $R^2 = CHO$; $R^7 = CO_2CH_3$
$R = (CH_2)_5CH_3$

e. $R^2 = CH{=}CH_2$; $R^7 = CO_2H$
$R = (CH_2)_4CH(CO_2Et)NH_2$

f. $R^2 = CH{=}CH_2$; $R^7 = CONHCH(CO_2Bu^t)CH_2CO_2Bu^t$
$R = (CH_2)_4CH(CO_2Et)NH_2$

g. $R^2 = CH{=}CH_2$; $R^7 = CONHCH(CO_2Bu^t)CH_2CO_2Bu^t$
$R = (CH_2)_4CH(CO_2Et)NH_2$

h. $R^2 = CH{=}CH_2$; $R^7 = CONHCH(CO_2Bu^t)CH_2CO_2Bu^t$
$R = (CH_2)_5CH_3$

i. $R^2 = CHO$; $R^7 = CONHCH(CO_2Bu^t)CH_2CO_2Bu^t$
$R = (CH_2)_5CH_3$

Some of the bacteriochlorins **2b**, **2f** and **4b** were evaluated for *in vivo* photosensitizing efficacy. The preliminary results are summarized in Table 1.

Table 1: Comparative *in vivo* Activity of Some Bacteriochlorins[§]

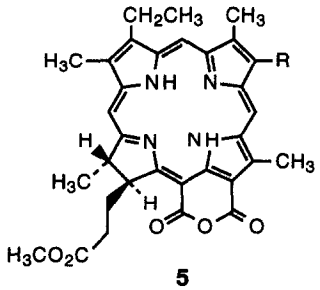
Compound	Dose mg/kg	Absorbance max	Time(h) between inj. and light treatment	%Tumor Response (days)[#]			
				1-2	7	21	30
2b	5.0	815	3.0	0	-	-	-
2f	5.0	815	3.0	100	100	80	80
4b	5.0	815	3.0	100	100	20	20

[§]4-6 mm diameter tumors were exposed to 75MW/cm^2 for 30 min to deliver 135 J/cm^2 from a tunable dye laser tuned to the maximum red absorption peak. [#]Non palpable tumors.

From the above results it can be seen that formylbacteriopurpurin-18 methyl ester **2b** was found to be biologically inactive at a dose of 5.0 mg/kg, when treated 3 h post i.v. injection of the drug. However, under similar treatment conditions, its aspartyl di-tert butyl ester derivative **2f** showed promising activity (80% tumor cure, day-30).

Bacteriochlorin **4b**, which still had propionic ester side chain at position-7 but in which the anhydride ring has been transformed into an imide ring system (R = L-lysine) showed much better activity (100% tumor cure, day 7, 20% tumor cure, day 30) than formylbacteriopurpurin-18 **2b** (no tumor cure at all). Comparing the biological results of bacteriopurpurins **2b** and **2f**, it is evident that aspartic acid substituents make a significant positive difference in biological activity. Thus, we believe that the anti-tumor activity of bacteriochlorin **4b** might further increase by replacement of the methyl ester with an aspartyl di tert-butyl ester side chain (**4g** or **4i**). The low activity of photosensitizer **2b** might be due to hydrolysis of the methyl ester to the corresponding carboxylic acid by esterases, which of course will make these photosensitizers extremely hydrophilic, and probably diminish their ability to be retained in the tumor cells.[11] Further biological studies with these photosensitizers at different doses and time intervals are in progress.

These results further confirm previously reported conclusions[12] that hydrophilicity/hydrophobicity properties make a remarkable difference in the localization characteristics of a photosensitizer in tumors, and should be considered as one of the most important requirements in designing an effective photosensitizer. In order to make the newly synthesized bacteriochlorins more hydrophobic, we decided to convert the *vic* -dihydroxy groups either into the corresponding ether or acetate derivatives or to protect them as acetone ketals. For initial studies, mesobacteriopurpurin **2c**, which was readily available, was used as a starting material. Attempts to convert the *vic* -hydroxy groups in **2c** to acetic esters or to protect them as ketal derivatives failed, and gross mixtures were obtained. Reaction of **2c** with a variety of alcohols in the presence of acid mainly gave the 4-vinylmesopurpurin-18 **5a** and 4-(1-alkoxyethyl)-mesopurpurin **5b** as major products.



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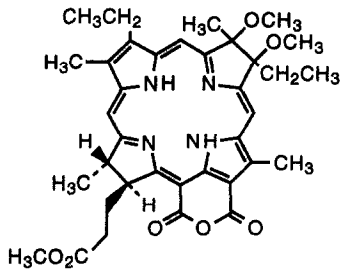

6

a. R = $CH{=}CH_2$
b. R = $CH(OH)CH_3$

The yield of 4-vinylmesopurpurin-18 **5a** was improved by reacting **2c** with a THF/HCl mixture. This is the first example of introducing a vinyl group at the opposite pyrrole subunit of the reduced pyrrole ring in a chlorin system. We have successfully used this methodology with methyl pyropheophorbide-a and mesochlorin-e_6 trimethyl ester and prepared the related 4-vinyl analogues. The replacement of a 4-ethyl with a 4-vinyl group, and subsequent Diels Alder reaction with a variety of dienophiles, has allowed us to prepare yet another new class of bacteriochlorin systems. These results will be reported elsewhere.[13]

Treatment of **2c** with silver oxide/methyl iodide, at room temperature and under nitrogen atmosphere gave the corresponding methyl ether derivative **6** in 60% yield. In the porphyrin, pheophorbide, and chlorin-e_6 series it has been shown that biological activity increased by increasing the length of an appended ether carbon chain. Attempts are currently being made to prepare the long carbon chain alkyl ether derivatives related to bacteriochlorin **6**. This methodology then will be used to prepare such alkyl ether derivatives of other long wavelength absorbing bacteriochlorins which have shown promising antitumor activity in preliminary screening. Further studies on other

bacteriopurpurin-18 related derivatives, and optimization of these compounds for use in PDT are in progress.

Acknowledgments:

This work was supported by grants from the National Institutes of Health (HL 22252, CA 55791), the National Science Foundation (CHE-93-05577) and Oncologic Foundation of Buffalo. Mass spectrometric analyses were performed at the Department of Biophysics, Roswell Park Cancer Institute, Buffalo.

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(*Received in USA* 14 *March* 1994; *accepted* 14 *April* 1994)