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Syntheses of Emeraldin and Purpurin-18 Analogs as Target-Specific Photosensitizers for Photodynamic Therapy

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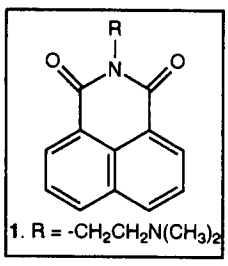
Abstract: *Efficient syntheses and mechanisms of the formation of some novel porphyrins and chlorins with cyclic anhydride, imide and isoimide rings bearing various N-substituents as possible target-specific photosensitizers for photodynamic therapy are presented. Porphyrins with such cyclic ring systems are named as "emeraldins" due to their intense green color. This class of compounds show a unique reverse etio- type absorption spectra.* © 1997 Published by Elsevier Science Ltd.

The DNA binding of various types of compounds has attracted an enormous interest not only as a topic in physical chemistry, but also in molecular pharmacology, medicinal chemistry and carcinogenesis.[1] Many of the current clinical, and experimental antitumor drugs are thought to act by disruption of nucleic metabolism at some level. The class of antitumor compounds that has received most development recently is the DNA binding agents, or "DNA adductors", that bind tightly but reversibly, to DNA by a combination of hydrophobic, electrostatic, hydrogen-bonding, and dipolar forces. At least three aspects of the binding of the drugs to DNA could be expected to influence biological activity; (i) mode of binding to the DNA, (ii) selectivity for A-T or G-C base pairs or specific sequences, and (iii) the kinetics of binding. Selectivity for specific sequences in the DNA may be of particular importance in the effectiveness of a drug, since, depending upon their sequence specificity, drugs could affect the activity of certain genes. Specific sequences are known to have profound effects on DNA conformation, and this in turn, can affect drug-binding properties.[2]

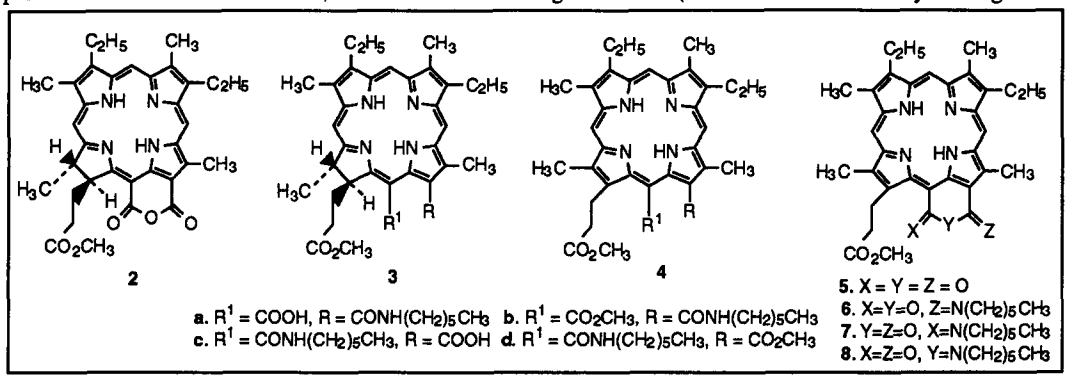
Among non-porphyrin systems, amonafide **1**, an imide derivative[3] and its structural analogs have been reported to inhibit DNA and RNA synthesis and bind to double-stranded DNA through intercalation. Some derivatives were also reported as anti-neoplastic agents *in vitro* as well as *in vivo*. Among all the derivatives, compounds with N,N-dimethylethyl substituent attached to the nitrogen of the cyclic imide ring were found to be the most effective. Encouraged by the results with these imide and isoimide analogs, we were prompted to investigate the effects of such cyclic structures in tetrapyrrolic systems. It has been shown that chlorins containing cyclic anhydride rings can be converted converted into isoimides and imides bearing N-alkyl substituents by leaving the solutions at room temperature for the extended period of time. However, the yields of the desired products were very low[4]. In order to compare the *in vivo* efficacy of chlorins and bacteriochlorins with anhydride, imide, or isoimide linkages[5]with porphyrins, we were interested in developing an efficient method for preparing porphyrins with similar fused ring systems. Extensive literature searches revealed that porphyrins with such cyclic imide and isoimide rings were, as yet, unknown.



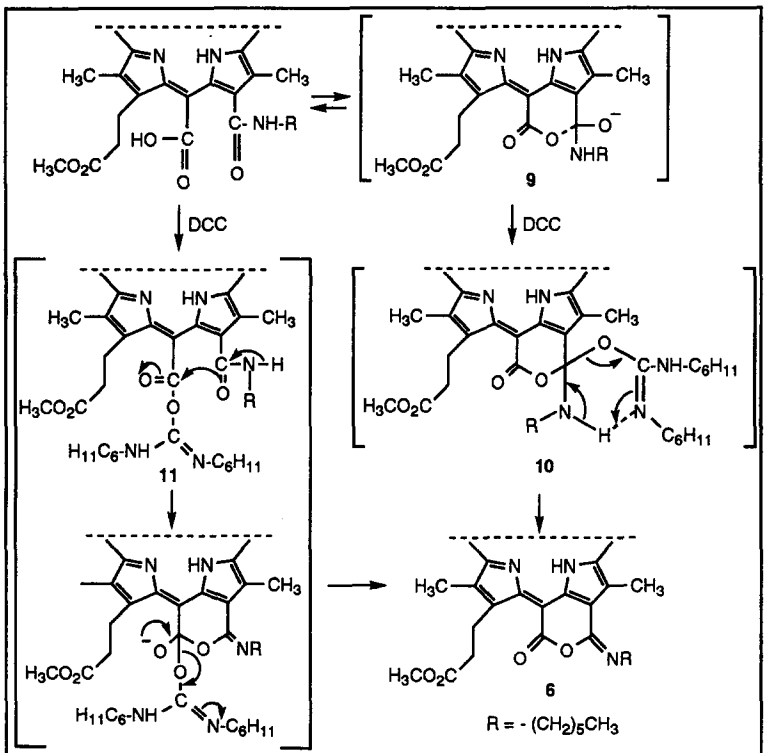
1. R = $-CH_2CH_2N(CH_3)_2$

Most chlorins and bacteriochlorins are susceptible to DDQ oxidation, and thus can be converted into the corresponding porphyrins in reasonable yields.[6] In our initial attempts to prepare porphyrins with anhydride and imide rings, mesopurpurin-18 methyl ester **2** was used as a substrate.[5] Unexpectedly, reaction of **2** with DDQ at room temperature did not oxidize the reduced pyrrole ring, and at elevated temperatures only decomposition

products were obtained. However, the related amide analogs **3a** and **3c** (a 6:1 mixture obtained by cleavage of the



anhydride ring with n-hexylamine) on DDQ treatment produced the corresponding bright red porphyrin **4a** and **4c** in 50% yield. The stability of **2** towards DDQ oxidation might be due to the electron-withdrawing nature of the fused cyclic anhydride ring adjacent to the reduced pyrrole ring (ring D) of the macrocycle. Intramolecular cyclization of the intermediate amide mixture with Montmorillonite clay (K-10) afforded porphyrin **5**, in 30% yield, as an intensely green-colored product.



Scheme 1. Possible mechanisms for the formation of fused isoimide ring by DCC method

In order to develop an efficient method for the synthesis of more stable porphyrins with imide rings, we thought it necessary to activate the carboxylic acid groups in **4a** and **4c**, which upon intramolecular base cyclization should produce the desired porphyrin. To our surprise, reaction of amides (**4a** and **4c**) with N, N'- dicyclohexyl carbodiimide (DCC) produced a mixture of green products, which was identified as isoimides **6** and **7** in a ratio of 6 to 1 (yield 65%). The isomeric mixture was separated into its individual isomers **6** and **7** which, upon reacting independently with methanolic KOH, generated porphyrin imide **8** in 80 % yield. Interestingly, reaction of porphyrins **4a** and **4c** (as a mixture) with diazomethane

and subsequent base-promoted cyclization also produced the related N-hexylimide derivative **8** in excellent yield.

The formation of N-substituted isoimide (e.g. **6**) by dehydration of intermediate amide (e.g. **4a**) with DCC, possibly proceeds *via* the mechanism outlined in Scheme 1. Donation of a proton from the intermediate amide to DCC could lead to a ring closed structure such as **8**. Further reaction with the DCC might lead to **9**,which could decompose *via* the indicated *quasi* six-membered ring transition state into N-substituted cyclic isoimide **6**. It can also be postulated that reaction of carbodiimide first generates the carbodiimide intermediate, which upon intramolecular cyclization would produce isoimide **11** and dicyclohexyl urea as a by product. Such six-membered ring formation has also been proposed in other aromatic systems.[7]

To our knowledge, these are the first examples of porphyrins containing cyclic anhydride, imide and isoimide rings. Due to their intense green color, we propose the generic name "emeraldin" for this novel class of porphyrins, following the tradition used in naming other porphyrin-type systems on the basis of their color in solution (e.g. sapphyrin, rubyrin).[8] The electronic absorption spectra of free base and metalated emeraldins were compared with those of other natural porphyrins. Interestingly, the absorption spectra of free base porphyrins were of the unique reverse etio- type (I>II>III>IV). The long wave length absorption band (band 1) was observed near 660-665 nm (ε 12,000- to 14,000), and was much more intense than the other Q bands. The "Soret" band, generally observed at 405-410 nm in natural porphyrins, was shifted in the emeraldin series to 430-438 nm. (Figure-1). Emeraldin-N-hexylimide **6** was converted into the corresponding Zn(II) complex in quantitative yield by following the standard metalation methodology; compared with the Zn(II) complex of natural porphyrins, where two Q bands are usually seen between 500 and 600 nm, a strong absorption at 654 nm with broad shoulder at 600 nm was observed. All emeraldins showed similar absorption spectra, and these types of spectra seems to be a characteristic of porphyrins with imide, isoimide and anhydride fused ring systems. The fluorescence spectra of all the emeraldins show an emission peak at 680 nm (excitation wavelength 660 nm). Thus, compared with protoporphyrin-IX (emission: 636 nm), a shift of 44 nm with decrease in intensity (fluorescence yields: 0.02-0.035) was observed. The fluorescence quantum yields of emeraldins were measured on a "per photon basis" relative to TPP ($\Phi_f = 0.11$) as the standard.[9]

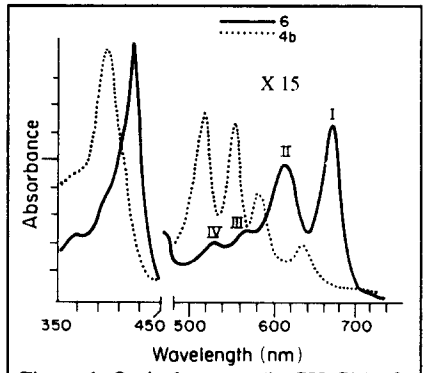
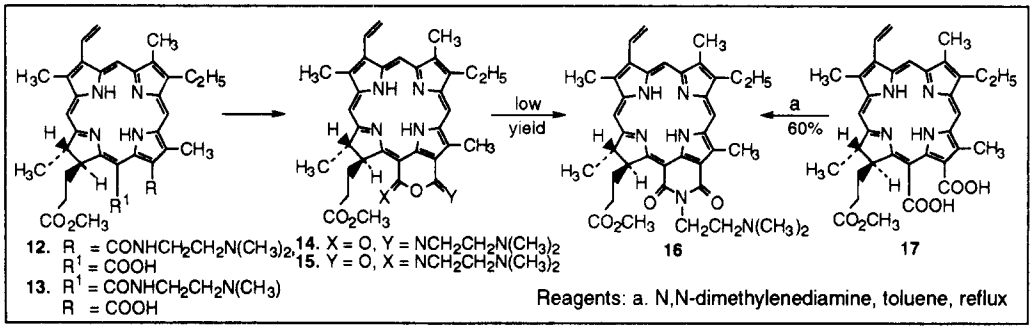


Figure 1. Optical spectra (in CH_2Cl_2) of emeraldin **6** and porphyrin **4b** obtained at the same molar concentrations.



Our initial attempts to prepare imide **16**, containing a N, N-dimethyl aminoethyl substituent (necessary for intercalating with DNA in the aminofide series) by following the method discussed above produced the desired compound in low yield. However, reaction of dicarboxylic acid **17**[6] with N,N-dimethylethylenediamine in refluxing toluene produced the corresponding imide in 60% yield. Surprisingly, when other alkylamines (e.g. n-

hexylamine) were used as reactants, the expected imides were not produced. On refluxing the reaction mixture, mainly decomposition products were obtained.

Recently, we have used certain cationic porphyrins prepared from the corresponding vinyl-extended Mannich adducts as substrates to understand the sequence specificity of the binding interactions with DNA using footprinting techniques. The initial studies suggest that these compounds show outside binding with DNA.[10] In order to extend these studies, we have also prepared a series of emeraldins from purpurin-18 methyl ester, containing a vinyl group. Manipulation of the vinyl substituent, (reaction with Eschenmoser's reagent, regioselective introduction of electron withdrawing groups, Diels-Alder reactions, dimerization, etc), will provide new porphyrins and chlorins. This work is currently in progress and will be published later.

All new compounds were characterized by ^{1}H NMR[11] and mass spectrometry (HRMS).

Acknowledgments:

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11. ^{1}H NMR (400 MHz, $CDCl_3$, 20 ^oC, δppm):

Emeraldin with isoimide ring **6**: 9.75, 9.64, 9.48 (each s, 3 H; *meso* -H), 5.03 (t, *J* = 7.9 Hz, 2H; hexylisoimide-*a*-CH_2), 4.28 (t, *J* = 7.6 Hz, 2H; 17*a*-CH_2), 3.89 (q, *J* = 7.8 Hz, 2H; 3*a*-CH_2), 3.85 (s, 3H; 12-Me), 3.75 (q, *J* = 7.8 Hz, 2H; 8*a*-CH_2), 3.48 (s, 3H; CO_2Me), 3.46 (s, 3H; Me), 3.28 (s, 3H; Me), 3.22 (t, *J* = 7.6 Hz, 2H; 17*b*-CH_2), 2.16 (m, 2H, hexylisoimide-*b*-CH_2), 1.86 (m, 2H, hexyiisoimide-*c*-CH_2), 1.76 (t, *J* = 7.8 Hz, 3H; 8-Me), 1.69 (t, *J* = 7.8 Hz, 3H; 17-Me), 1.52 (m, 4H, 6-hexylisoimide-*d,e*-CH_2CH_2), 0.99 (t, *J* = 7.5 Hz, 3H, hexylisoimide-*f*- CH_3), -0.8, -1.2 (each broad s, 1H, NH).

Emeraldin with isoimide ring **7**: 9.70, 9.61, 9.44 (each s, 3 H; *meso* -H), 5.05 (t, *J* = 7.9 Hz, 2H; hexylisoimide-*a*-CH_2), 4.22 (t, *J* = 7.6 Hz, 2H; 17*a*-CH_2), 3.79 (q, *J* = 7.8 Hz, 2H; 3*a*-CH_2), 3.83 (s, 3H; 12-CO_2Me), 3.76 (q, *J* = 7.8 Hz, 2H; 8*a*-CH_2), 3.51 (s, 3H; CO_2Me), 3.49 (s, 3H; Me), 3.29 (s, 3H; Me), 3.29 (t, *J* = 7.6 Hz, 2H; 17*b*-CH_2), 2.18 (m, 2H, hexylisoimide-*b*-CH_2), 1.80 (m, 2H, hexylisoimide-*c*-CH_2), 1.78 (t, *J* = 7.8 Hz, 3H; 8-Me), 1.72 (t, *J* = 7.8 Hz, 3H; 17-Me), 1.52 (m, 4H, 6-hexylisoimide-*d,e*-CH_2CH_2), 0.99 (t, *J* = 7.9 Hz, 3H, hexylisoimide-*f*- CH_3), -0.6, -1.0 (each broad s, 1H, NH)

Emeraldin with imide ring **8**: 9.78, 9.66, 9.58 (each s, 3 H; *meso* -H), 4.53 (t, *J* = 7.9 Hz, 2H; hexylimide-*a*-CH_2), 4.46 (s, 3H; 13-CO_2Me), 4.22 (t, *J* = 7.8 Hz, 2H; 17*a*-CH_2), 3.94 (q, *J* = 7.8 Hz, 2H; 17*a*-CH_2), 3.70 (s, 3H; 12-Me), 3.78 (q, *J* = 7.8 Hz, 2H; 8*a*-CH_2), 3.64 (s, 3H; CO_2Me), 3.48 (s, 3H; Me), 3.46 (s, 3H; Me), 3.38 (s, 3H, Me), 3.26 (t, *J* = 7.8 Hz, 2H; 17*b*-CH_2), 2.19 (m, 2H, hexylimide-*b*-CH_2), 1.89 (m, 2H, hexylimide-*c*-CH_2), 1.75 (t, *J* = 7.5 Hz, 3H; 8-Me), 1.69 (t, *J* = 7.5 Hz, 3H; 17-Me), 1.49 (m, 4H, 6-hexylimide-*d,e*-CH_2CH_2), 0.98 (t, *J* = 7.9 Hz, 3H, hexylimide-*f*- CH_3), -0.7, -1.1 (each broad s, 1H, NH).

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