ORGANIC LETTERS

2002
Vol. 4, No. 12
2013−2016

Photobleaching of Compounds of the 5,10,15,20-Tetrakis(*m*-hydroxyphenyl)-porphyrin Series (m-THPP, m-THPC, and m-THPBC)

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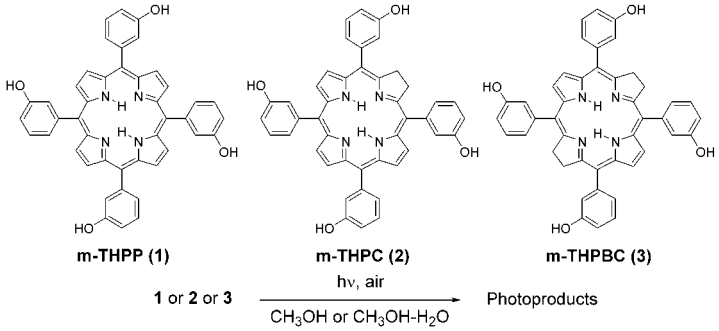
Received March 8, 2002

ABSTRACT



5,10,15,20-Tetrakis(*m*-hydroxyphenyl)porphyrin (m-THPP) yielded novel quinonoid porphyrins upon irradiation in aqueous methanol. True photobleaching was observed for 5,10,15,20-tetrakis(*m*-hydroxyphenyl)chlorin (m-THPC) and 5,10,15,20-tetrakis(*m*-hydroxyphenyl)bacteriochlorin (m-THPBC) under the same conditions; several fragmentation products (imides, methyl *p*-hydroxybenzoate, dipyrrin derivatives) were recognized.

Photodynamic therapy (PDT) is emerging as a treatment for a variety of conditions, but particularly for cancer and wet age-related macular degeneration. It requires a combination of oxygen, visible light, and a drug (a photosensitizer), which causes damage to living tissue.[1] The 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin series[2] (m-THPP (**1**), m-THPC (**2**), and m-THPBC (**3**)) includes some of the most potent photosensitizers discovered to date. The chlorin, m-THPC (**2**) (FOSCAN, temoporfin), has recently received regulatory approval in the EU for the treatment of head and neck cancer.[3] In photochemistry and photobiology, photobleaching is defined as the loss of absorption or emission intensity[4] caused by light. Photobleaching of porphyrin analogues most commonly used in PDT can occur by photooxidation or photoreduction processes, the former being more common. Two types of irreversible photobleaching process leading to chemical change of the chromophore are encountered:[5] (i) photomodification, where the chromophore is retained in a *modified* form; (ii) true photobleaching, where chemical changes are deep seated and result in small fragments that no longer have appreciable absorption in the visible region. Photobleaching of photosensitizers during PDT has been observed both in cells *in vitro* and in skin *in vivo* using the fluorescence mode of observation, both directly and after pigment extraction.[6] While photobleaching might be thought to be a disadvantageous property in a tumor photosensitizer (in that the source of the active species is being destroyed),

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10.1021/ol025842c CCC: $22.00
Published on Web 05/23/2002

it is possible to envisage potential benefits, for example, in limiting the damage to healthy tissue by working at the appropriate dose of photosensitizer.[5,6] In recent years, photobleaching has received increasing attention. However, most of the reports to date are kinetic studies and often little is known about the structures of the photoproducts.[5]

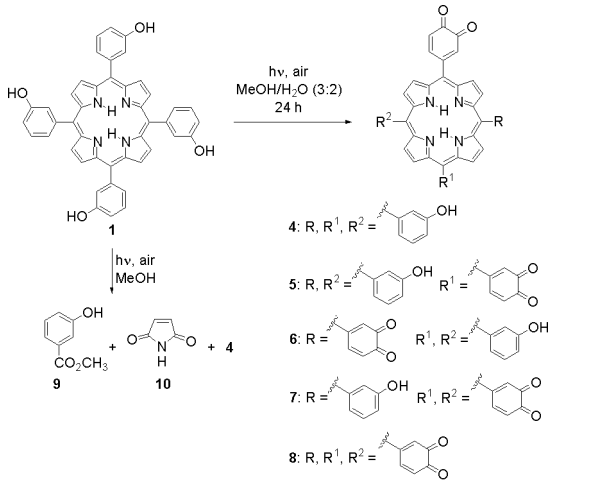
The first comparative kinetic study[7] of the photobleaching of the m-THPP series *in vitro* using laser irradiation was recently reported. True photobleaching was observed for m-THPC (**2**) and m-THPBC (**3**), but the porphyrin (**1**) reacted more slowly, with photomodification. Evidence was found for the involvement of singlet oxygen in all three systems. We now wish to report our preparative studies using broad band UV−vis radiation.

m-THPP (**1**) was irradiated (two Philips MLU 300 W lamps) for 24 h in methanol−water (60:40) in a water-cooled photoreactor.[8] The absorbance decay at band IV ($\lambda_{max} = 512$ nm) was 20%, band III ($\lambda_{max} = 546$ nm) intensity decreased 22%, and the absorbance of band II ($\lambda_{max} = 587$ nm) decayed 8%. The methanol of the irradiated solution was then removed, and the resulting aqueous suspension was extracted with ethyl acetate, leaving a colorless water layer. Preliminary analysis of the organic layer by TLC on silica showed the presence of four major components, m-THPP (**1**), two brown bands *less* polar than **1**, and a purple base line. The least polar brown band was found to contain four minor components separated by preparative TLC. The second brown band was isolated in larger amount (25%) as puce solid **4** after flash chromatography; it was followed by m-THPP (**1**, 32% recovery). The porphyrin nature of the five photoproducts was immediately apparent from the UV−vis spectra.

The measured accurate mass and elemental analyses for the major photoproduct **4** were consistent with the molecular formula $C_{44}H_{28}N_4O_5$ (M + H 693.2084, calcd 693.2138), a compound containing an extra oxygen when compared with m-THPP (**1**). The UV−vis spectrum of **4** showed bands at 413, 510, 547, 588, and 645 nm. Qualitatively, **4** showed little fluorescence on the TLC plate (366 nm), in contrast to the bright red fluorescence observed for m-THPP (**1**). This difference in fluorescence was confirmed when the emission spectra ($\lambda_{exc} = 423$ nm) of m-THPP (**1**) and **4** were recorded at nearly the same concentration ($c = 1.55 \times 10^{-6}$ and 1.7×10^{-6} M, respectively). The fluorescence of m-THPP (**1**) at 648 nm was three times more intense than that of the photoproduct. It has been shown that *meso*-benzoquinonylporphyrins have their fluorescence diminished considerably,[9] so **4** was tentatively regarded as a benzoquinonylporphyrin. The quinonoid nature of this porphyrin was confirmed after extensive spectroscopic studies (IR, ^{1}H and ^{13}C NMR).[8]

Three benzoquinone isomers of **4** are possible, namely, two *o*- and one *p*-benzoquinone. The 2,3-*o*-benzoquinone was

Scheme 1. Photobleaching of m-THPP (**1**)



rapidly ruled out on the basis of ^{1}H NMR evidence. To distinguish between the 3,4-*o*-benzoquinone and the 2,5-*p*-benzoquinone is not simple on the basis of spectroscopic evidence alone. We then embarked in the synthesis of a series of 5-(dihydroxyphenyl)-10,15,20-tris(3-hydroxyphenyl)porphyrins **11**−**14** (Scheme 2).[10]

With these porphyrins in hand, we proceeded to reduce the photoproduct. When **4** was treated with $NaBH_4$ (MeOH, 2.5 h), the starting material disappeared and a new material was formed. On the TLC plate, the new porphyrin was more polar than the photoproduct and m-THPP (**1**) and it showed a strong red fluorescence as did porphyrins **11**−**14**, in contrast to the weak brown fluorescence shown by **4**. This new material showed a molecular ion at *m*/*z* 695 (695.2264) which corresponded to the molecular formula $C_{44}H_{30}N_4O_5$ + H (695.2294). Thus, the reduction of photoproduct **4** to yield the corresponding dihydroxyphenylporphryrin had been achieved. In the ^{1}H NMR spectra of **11**−**14**, each benzenoid region is very different and can be used as a fingerprint.[8] The reduction product from the photoproduct was only similar in the benzenoid region to 5-(3,4-dihydroxyphenyl)-10,15,20-tris(3-hydroxyphenyl)porphyrin (**11**) and the two samples were undistinguishable on the TLC plate. Furthermore, the dehydrogenation of **11** with DDQ yielded a mixture of mobile porphyrins: the products showed reduced fluorescence on TLC under 366 nm light, and one of them was identical with authentic material **4** on mixed TLC. However, it was not possible to isolate a pure sample for further analysis. Although we have attempted to grow crystals of **4**, they have been unsuitable for X-ray analysis. Our conclusion is that the structure which best fits with these experimental results is 5-(3,4-benzoquinonyl)-10,15,20-tetrakis(3-hydroxyphenyl)porphyrin (**4**) (Scheme 1).

The presence of additional oxygen atoms in the remaining four photoproducts compared to the starting material **1** was

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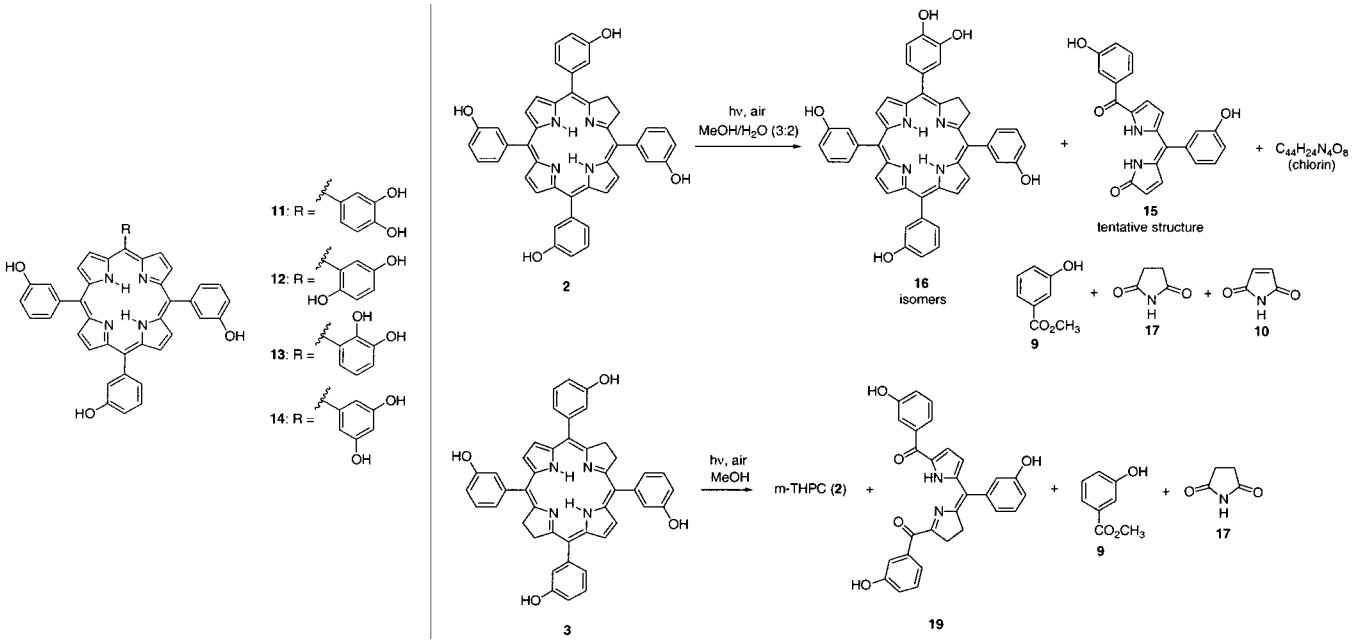
(8) For experimental procedures and characterization of new compounds, see Supporting Information.

(9) (a) Chin, A.; Dalton, J.; Milgrom, L. R. *J. Chem. Soc., Perkin Trans. 2* **1982**, 707. (b) Dalton, J.; Milgrom, L. R. *J. Chem. Soc., Chem. Commun.* **1979**, 609. (c) D'Souza, F.; Deviprasad, G. R.; Hsieh, Y. Y. *J. Chem. Soc., Chem. Commun.* **1997**, 533. (d) Deviprasad, G. R.; Keshavan, B.; D'Souza, F. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3133.

(10) Novel porphyrins **11**−**14** were prepared by the cross condensation in propionic acid of 3-methoxybenzaldehyde (3 equiv) and the appropiate dimethoxybenzaldehyde (1 equiv), followed by deprotection of the methyl ethers to yield the phenols using BBr_3. See details in Supporting Information.

Scheme 2. Porphyrins **11**−**14** and photobleaching of m-THPC (**2**) and m-THPBC (**3**)



proved by mass spectroscopy. The mass spectra (FAB) showed that **5** and **6** had two extra oxygen atoms. The other two minor photoproducts **7** and **8** appeared to have three and four extra oxygen atoms, respectively (Table 1).

Table 1. MS(FAB) for Benzoquinonylporphyrins **5**−**8**

5: $C_{44}H_{26}N_4O_6$ + 5H	**6**: $C_{44}H_{26}N_4O_6$ + 5H	**7**: $C_{44}H_{24}N_4O_7$ + 7H	**8**: $C_{44}H_{22}N_4O_8$ + 7H
711.2270[a]	711.2198[a]	727.2170[a]	741.1960[a]
711.2244[b]	711.2244[b]	727.2193[b]	741.1960[b]

[a] Found. [b] Calculated.

However, the molecular ions reported showed extra hydrogen atoms above those required by the expected quinonoid formula. Reduction of quinones is often observed during the mass spectrometric process, and this appears to be occurring.[11] The mass of **6** was also measured by the APCI technique, and the quinone rings did not appear to be reduced, since *m*/*z* 707 (M + H) was measured.

Several features of the UV−vis spectra of **4**−**8** in methanol are of interest: the Q-bands were broadened; the original band III (λ_{max} = 546 nm) of m-THPP (**1**) decreased when one benzoquinone ring (in **4**) was present and disappeared for **5**−**8**; band IV was shifted to the blue ($\Delta\lambda$ = 2−9 nm); and bands II and I moved to the red $\Delta\lambda$ = 3 and 9 nm, respectively. The same type of behavior has been reported for a series of 2,5-benzoquinonylporphyrins.[9] Photoproducts **5**−**8** also showed little fluorescence on the TLC plate when irradiated with 366 nm light. Further spectroscopic studies (IR, ^{1}H and ^{13}C NMR) led to the formulation of these four compounds as benzoquinonylporphyrins **5**−**8** (Scheme 1).[8]

Kinetic studies[7] show that the photooxidation of m-THPP (**1**) is slower in methanol than in methanol−water (60:40), and the same was observed in these preparative studies. After 41 h irradiation in methanol,[8] the absorbance decay of band IV (λ_{max} = 512 nm) was only 16% and the recovery of m-THPP (**1**) was 53%. In addition to m-THPP (**1**), small amounts of **4** (5%) were obtained. A number of fractions, more polar than **1**, were also isolated and appeared to be porphyrins according to their UV−vis spectra. Due to the small quantities formed, their structures were not studied further. After 140 h, methyl 3-hydroxybenzoate (**9**, 2.5%) and maleimide (**10**, 1.1%) were identified as the components of a mobile fraction by ^{1}H NMR, MS(EI), and GLC (Scheme 1).[8]

Solutions of m-THPC (**2**) in methanol or methanol−water (60:40) were irradiated for 23 and 15 h, respectively.[8] The decay of absorbance was observed throughout the whole region (450−800 nm). As observed during the kinetic studies,[7] the reaction occurred much more slowly in methanol than in methanol−water (60:40). After 23 h of irradiation in methanol, preparative TLC of the crude yielded m-THPC (**2**, 46%) as the major fraction. A green base line and several minor bands were also observed, but they accounted for less than 10% of recovered material. However, a much more extensive photodegradation of m-THPC (**2**) was effected under the same experimental conditions in methanol−water (60:40). In only 15 h, the absorbance decay of band I (λ_{max} = 749 nm) was 78%. m-THPC (**2**, 10%) was recovered

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together with eight other fractions after preparative TLC on silica (Scheme 2).

The two most mobile fractions were white solids. The major one was a mixture of two small fragments which were identified by ^{1}H NMR, MS(EI), and GLC as methyl 3-hydroxybenzoate (**9**, 1.5%) and maleimide (**10**, 1.2%). The minor white solid was identified by GLC (comparison with authentic sample) as succinimide (**17**, 0.6%). Two yellow fragments, which were less polar than m-THPC (**2**), were also isolated. The major one was obtained as a yellow-orange solid. One peak in the MS(FAB) was found at *m*/*z* 373, which can be ascribed to the molecular formula $C_{22}H_{15}N_2O_4$ + 2H. A second more mobile yellow material was also isolated in still smaller amounts. The UV−vis spectra of both substances showed two main bands at 314 and 400 nm which suggested that these materials could be dipyrrolic compounds. Pyrromethenones with no substituents in the carbon bridge show bands at 264 and at 400 nm.[12a] 5-Phenyl-4,6-dipyrrin also shows absorption bands in this region, at 354 and 466 nm.[12b] Some assignments were made from the ^{1}H NMR of the first fraction and tentatively led us to formulate this material as **15**.

Two more major fractions were isolated. Both materials were colored, and their UV−vis spectra showed that they were chlorins.[8] The least polar chlorin was isolated as a dark brown solid: the pigmented spot showed little fluorescence on the TLC plate under 366 nm light. The MS(FAB) spectrum showed a major peak at *m*/*z* 744 which could correspond to a molecular formula such as $C_{44}H_{32}N_4O_8$ (four more oxygens than m-THPC (**2**)). Since this chlorin was more mobile than **2**, it was thought that it could have had the four phenol rings transformed into benzoquinones. The formation of an unidentified species which showed an ion at *m*/*z* 744 in the mass spectrum has also been reported by Bezdetnaya et al.[13] from the photooxidation of **2** in ethanol. The last major fraction was another chlorin which was more polar than m-THPC (**2**). The MS(FAB) spectrum showed a major peak at *m*/*z* 697 (found 697.2493) which corresponded to the molecular formula $C_{44}H_{32}N_4O_5$ + H (calcd 697.2451) (one more oxygen than m-THPC (**2**)). The ^{1}H NMR was very complex, suggesting the presence of several isomers of **16**. Such a photoproduct was also obtained by Jones et al.[14] during irradiation of **2** in methanol.

To complete the series, the photobleaching of m-THPBC (**3**) in methanol was studied.[8] Decay of absorbance throughout the whole spectrum was observed in just 4.5 h, indicating true photobleaching.[7] The photobleaching of bacteriochlorin **3** was by far the most rapid process studied in this series. After irradiation, the methanol was removed and the crude solid was directly purified by preparative TLC. There were only three major bands in the plate (Scheme 2).

The most mobile fraction proved to be a mixture. It was isolated as a white solid and analyzed by GLC and MS(EI). Methyl 3-hydroxybenzoate (**9**) and succinimide (**17**) were identified as the components of the mixture. The total recovered yield never exceeded 5%. Maleimide (**10**) was not detected. The second fraction was an orange red pigment which was less mobile than **9** and **17** but less polar than m-THPBC (**3**). The color suggested that this pigment was a linear pyrrolic pigment and it was formulated as 7,8-dihydro-1,9-bis(3-hydroxybenzoyl)-5-(3-hydroxyphenyl)pyrromethene (**19**). The MS(FAB) spectrum showed a major peak at 479 (found 479.1598) which corresponded to the molecular formula $C_{28}H_{22}N_2O_5$ + H (calcd 479.1607). The UV−vis spectrum showed broad bands at 332 and 429 nm, which are characteristic of 5-phenylpyrromethenes.[12b] The ^{1}H NMR and COSY spectra also provided support for structure **19**.[8]

The third product isolated was m-THPC (**2**). The chlorin **2** was already in the starting material as an impurity, the content being ca. 5−10% (determined by ^{1}H NMR). During the short period used for the photobleaching of the bacteriochlorin **3**, m-THPC (**2**) is expected to suffer little photobleaching: it was obtained in 4−8% yields. In this preparative study, it was not possible to establish by isolation of material if photooxidation of bacteriochlorin to chlorin was occurring (initial content ca. 5−10%; isolated 4−8%). To prove the formation of m-THPC (**2**), the photobleaching of m-THPBC (**3**) was followed by ^{1}H NMR spectroscopy.[15] The peaks corresponding to m-THPC (**2**) gradually increased in intensity (e.g., the area of the peak at δ 8.40 ppm of **2** increased ca. 50%). Thus, the formation of m-THPC (**2**) upon irradiation of aerated solutions was confirmed. When the experiment was carried out excluding the air, the ^{1}H NMR spectra were unchanged, confirming that oxygen was essential for the photobleaching to occur.

In conclusion, the first example of the photooxidation of phenolic porphyrin **1** to give benzoquinonylporphyrins is reported.[16] The corresponding chlorin **2** and bacteriochlorin **3** are photooxidized more readily, and cleavage products include orange compounds regarded as dipyrrin derivatives. Small amounts of colorless fragmentation products (imides, methyl *m*-hydroxybenzoate) are detected in each case, and **3** gives a small but detectable yield of **2**.

Acknowledgment. G.M. is indebted to the University of London Postgraduate trust for the award of a Laura de Saliceto Cancer Research Studentship (1996−1998). Spectroscopic support from ULIRS (School of Pharmacy, Queen Mary College). EPRSC (Swansea) is acknolwedged.

Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is free of charge via the Internet at http://pubs.acs.org.

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(14) Jones, R. M.; Wang, Q.; Lamb, J. H.; Djelal, B. D.; Bonnett, R.; Lim, C. K. *J. Chromatog. A* **1996**, *722*, 257.

(15) Using two Philips MLU 300 W lamps, 17 mW/cm^2, m-THPBC (**3**) ($c = 8.8 \times 10^{-3}$ M), was irradiated in air-saturated CD_3OD (0.7 mL). The NMR tube was placed in a water bath (constant flow of water) and irradiated. At intervals, the tube was removed from the bath and the ^{1}H NMR spectrum was recorded.

(16) The formation of *p*-benzoquinones from phenols mediated by singlet oxygen has been previously reported, see: Saito, I.; Matsuura, T. In *Singlet Oxygen, Organic Chemistry, Vol. 40*; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York 1979; p 511.