Tetrahedron Letters, Vol.31, No.51, pp 7399-7402, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

SYNTHESES, STABILITY, AND TUMORCIDAL ACTIVITY OF PORPHYRIN DIMERS AND TRIMERS WITH ETHER LINKAGES

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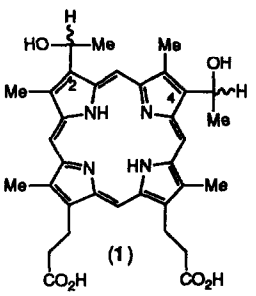
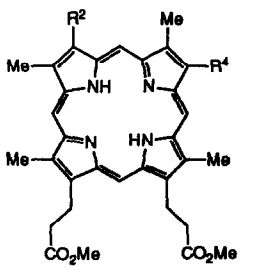
Abstract: The first syntheses of a series of regiochemically pure porphyrin dimers and trimers with ether linkages which are structurally related to Photofrin-II® are described. Stability of these oligomers was investigated using variable temperature proton NMR spectroscopy, and preliminary biological results reveal that manupulation of peripheral substituents causes a remarkable difference in photosensitizing ability in vivo.



(1)



(2) R^2 = H; R^4 = CH(OH)Me
(3) R^2 = CH(OH)Me; R^4 = H
(4) R^2 = CH(OH)Me; R^4 = CO.Me
(5) R^2 = CO.Me; R^4 = CH(OH)Me
(6) R^2 = R^4 = CO.Me
(7) R^2 = CO.Me; R^4 = CH=CH_2
(8) R^2 = CH=CH_2; R^4 = CO.Me
(9) R^2 = CO.Me; R^4 = CH(Br)Me
(14) R^2 = R^4 = CH(OH)Me
(15) R^2 = R^4 = CH(Br)Me
(17) R^2 = R^4 = CH=CH_2

Photodynamic therapy (PDT) depends upon the ability of a photosensitizer to accumulate in malignant tissue at levels higher than in surrounding normal tissue.[1] Photofrin II®, prepared from hematoporphyrin (**1**),[2,3] is currently in phase III clinical trials for the treatment of a variety of tumors by PDT. Despite numerous studies with Photofrin II® little is known about its mode of action, principally due to its complex chemical composition.[4,5] In our efforts to characterize Photofrin II® and to delineate structure/activity relationships of possible components, we and others have reported that porphyrin dimers with ester linkages are unstable under physiological conditions.[6,7] We have also reported syntheses of porphyrin dimers with carbon-carbon linkages and observed that the dimers prepared from 4-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**2**) are more active than those prepared from the regioisomer, 2-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**3**).[8]

We have previously synthesized ether linked hematoporphyrin dimers and trimers as a mixture of regio- and stereo- isomers, starting from a mixture of 4-acetyl-2-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**4**) and its 2-acetyl-4-(1-hydroxyethyl) derivative (**5**).[9] This mixture was prepared by the partial reduction of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**6**). Our goal is to prepare and study regiochemically pure dimeric and trimeric photosensitizers related to Photofrin II® which has been facilitated by our recent one step synthesis (and separation)[9] of (**4**) and (**5**) from hematoporphyrin dimethyl ester. The monoacetyl monovinyl porphyrins (**7**,**8**) were prepared from the corresponding monoacetyl mono(1-hydroxyethyl) derivatives (**4**,**5**) by following the reported procedure.[10] The present paper describes the syntheses, stability and biological activity of regiochemically pure ether-linked photosensitizers related to Photofrin II®.

The target dimers were prepared by following two different strategies. In our first

approach, 2-acetyl-4-(1-hydroxyethyl)deuteroporphyrin dimethylester (**5**) was reacted with methane sulfonyl chloride (< -70°C) for 1h followed by lithium bromide. The resulting bromo compound (**9**) was reacted with starting porphyrin (**5**) and the dimer product (**10a**) was isolated in 32% yield. Reduction of the diacetyl dimer (**10a**) with $NaBH_4$ produced the hematoporphyrin dimer (**10b**) in 80% yield. In the second approach, HBr gas was bubbled into the solution of porphyrin (**5**) in dry dichloromethane for 5 sec and solvent was removed under high vacuum (0.1mm Hg). Porphyrin (**5**) was added below 5°C and gave the diacetyl dimer (**10a**) in 35% yield. Other porphyrin dimers (total 14 dimers, Figure 1) were prepared by following the same methodology, using appropriate porphyrins as the starting materials.

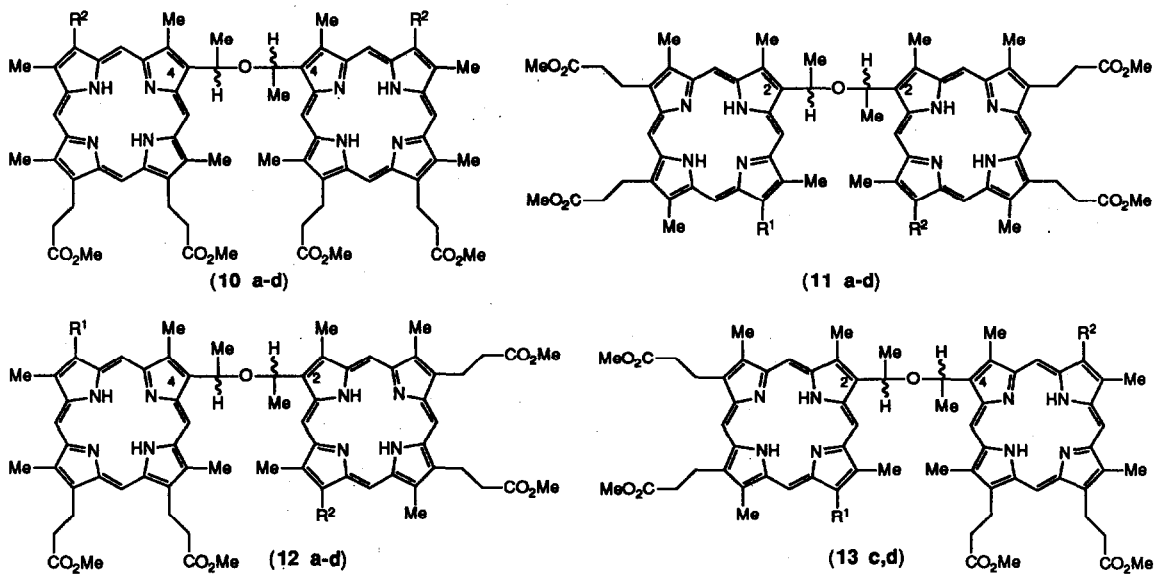
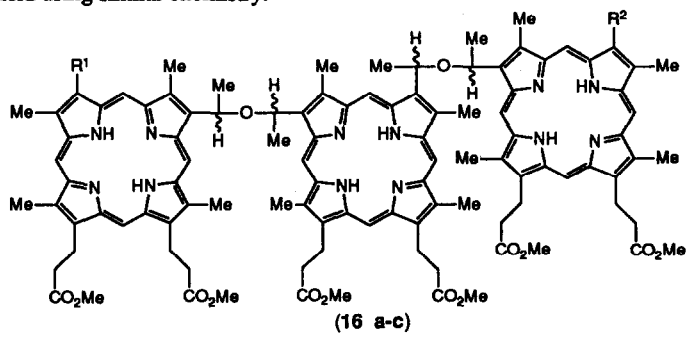
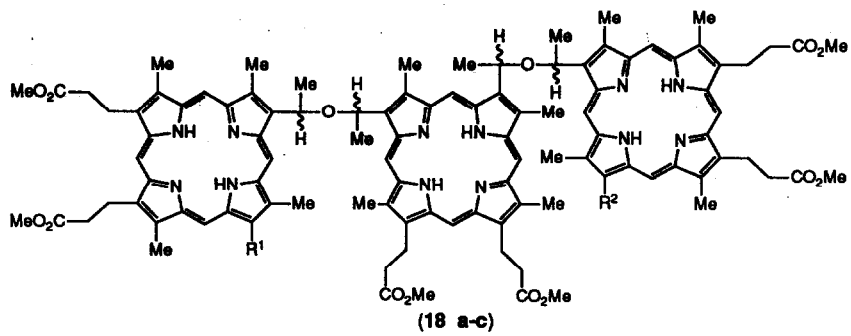


Figure 1: Structures of synthetic dimers

(a) R^1=R^2=CO.Me; (b) R^1=R^2=CH(OH)Me; (c) R^1=CO.Me, R^2=CH=CH_2; (d) R^1=CH(OH)Me, R^2=CH=CH_2.

For the preparation of the porphyrin trimers, hematoporphyrin dimethyl ester (**14**) was first converted into its dibromo analogue (**15**) by treatment with HBr gas and then condensed with two equiv of 2-acetyl-4-(1-hydroxyethyl)deuteroporphyrin dimethyl ester (**5**) to give trimer (**16a**). Thus a series of trimers (6 trimers, Figure 2) was prepared in 30 to 35% yield using similar chemistry.





(18 a-c)

Figure 2: Structures of synthetic trimers
(a) R^1=R^2=CO.Me; (b) R^1=R^2=CH(OH)Me; (c) R^1=R^2=CH=CH_2.

There is speculation concerning the stability of the various components of Photofrin II®, which appears to consist of a mixture of dimers and higher oligomers containing ester, ether, and possibly carbon-carbon linkages. Dimers with ester linkages were found to be unstable and readily cleaved,[6] but dimers with carbon-carbon linkages were found to be very stable at high temperatures.[11] In order to investigate the stability of the new ether linked dimers reported here we have employed variable temperature proton NMR spectroscopy.

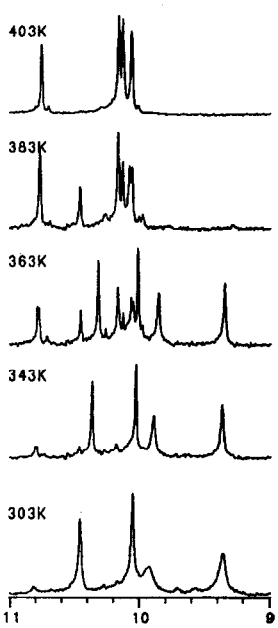


Figure 3: 300 MHz variable temperature proton NMR study of dimer (**10a**) (meso-proton region only) in $CDCl_2CDCl_2$ solvent.

Thus, dimer (**10a**) (Figure 3) and trimer (**16a**) (not shown) were dissolved separately in d_2-tetrachloroethane and spectra were recorded at various temperatures. Figure 3 shows the low-field region in the proton NMR spectrum of dimer (**10a**) at temperatures between 303 and 403 K. It is evident that there is cleavage of the ether linkages when the dimer (and trimer, not shown) are heated in tetrachloroethane solution. The resulting monomers also undergo dehydration i.e. (1-hydroxyethyl) ⇒ vinyl. Thus, dimer (**10a**) produced 2-acetyl-4-vinyldeuteroporphyrin IX dimethyl ester (**7**) as the sole product (Figure 3), and the presence of the 2-acetyl-4-(1-hydroxyethyl)porphyrin as an intermediate in this process is indicated[9] by the meso-proton resonance at approx. 10.4 ppm in the 363 and 383 K spectra (Figure 3). Trimer (**16a**) produced 2-acetyl-4-vinyldeuteroporphyrin IX dimethyl ester (**7**) and protoporphyrin IX dimethyl ester (**17**) in the ratio of 2 to 1. At 37°C, which is very close to tumor temperature, some cleavage of the ether linkages was observed. As it is well known that ethers are cleaved by acid, and because tetrachloroethane (the NMR solvent) may contain traces of acid, these results may at first seem to be of limited value. However it has been reported that tumors are acidic,[12] so there is a possibility that the ether linked oligomers of Photofrin II® might be cleaving in the tumor at body temperature. When the variable temperature experiments were repeated using pyridine as a solvent, no cleavage products were observed, showing that the ether linkages are thermally stable. Further model work as well as *in vivo* NMR studies with these components are in progress and will be reported in due course.

Preliminary *in vivo* biological testing for tumorcidal activity was performed as described previously and results were compared with Photofrin II®.[13,14] All the isomers of hematoporphyrin dimer (**10b, 11b, 12b**) were found to be inactive at a dose of 4.2 mg/Kg compared to Photofrin II®. Monohydroxyethyl monovinyl dimer (**10d**) showed activity

(comparable to Photofrin II® at 4.2 mg/Kg) with almost no skin phototoxicity after day 2 at a dose of 1.0 mg/kg if both heat (42°C) and light were used. Surprisingly, if only light was used the dimer was found to be inactive, even at a dose of 4.2 mg/kg. In the trimer series, the hematoporphyrin trimer (**16b**) was found to have similar tumorcidal actvity and skin phototoxicity compared to Photofrin II®. These photosensitizers, as methyl esters or as carboxylic acids, gave the same tumor response.[14] The biological studies with other dimers and trimers are in progress and will be reported later.

Acknowledgments: K.M.S. thanks the National Institutes of Health (HL 22252) and the National Science Foundation (CHE-86-19034) for support of this research. C.J.M. is grateful to the Fulbright Commission for a Travel Scholarship. We are grateful to Adam Sumlin (Roswell Park) for his assistance with the biological studies.

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14. For determination of biological activity, photosensitizers with methyl esters were dissolved in Tween 80 and then diluted with ten fold normal saline solution for the preparation of the injected solution. We have found in other studies that Tween 80 does not result in uptake/retention of inactive porphyrins like hematoporphyrin or hinder the activity of active material, e.g. Photofrin II®.

(Received in USA 4 September 1990)