Porphyrin Derivatives: Synthesis and Potential Applications

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Porphyrin-porphyrin and porphyrin-chlorin dimers have been synthesized and evaluated for their photophysical and *in vivo* photodynamic therapy properties. Two of them can become potential new photosensitizers. Study of the reactivity of *meso*-tetraaryl porphyrins, as dienophiles, in Diels-Alder transformations and as dipolarophiles in 1,3-dipolar cycloadditions, has been undertaken. New synthetic methodologies for certain chlorin, bacteriochlorin and isobacteriochlorin type macrocycles, with potential biological significance, have been established.

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Introduction.

Porphyrin derivatives play several vital functions in nature. The structure elucidation in conjunction with the establishment of adequate synthetic routes, and the understanding of their modes of action have led to intensive studies carried out by many research groups with fascinating results. More recently interdisciplinary studies are being performed with porphyrinic macrocycles aiming to find important applications for such compounds. The results obtained have pointed out for significant uses of these compounds in catalysis [1], as sensors [2] or biocides [3], as new electronic materials [4] and in medicine [5].

The use in medicine is mainly concerned with the detection and therapy of cancer cells. The former relies on the fluorescence properties of such xenobiotics and the latter on the properties of their triplet excited states to participate in electron transfer, hydrogen abstraction or interaction with oxygen in the formation of singlet oxygen (type I and II photoreactions). The use of a photosensitizer, visible light and oxygen to selectively destroy certain living cells is a process known as photodynamic therapy (PDT); in such way, the formation of singlet oxygen is of especial significance *in vivo*. PDT already plays a landmark in the treatment of neoplastic lesions, currently being in use in several countries. However the marketed formulations (the hematoporphyrin derivative (HpD) or commercial variants, such as Photofrin) are complex mixtures of monomers and oligomers. The structures of the most active components have not yet been elucidated, but some evidence points to their dimeric or oligomeric nature. Furthermore they are not very selective, causing skin sensitivity for some weeks, and have weak absorption bands in the red (at *ca.* 630 nm). Such disadvantages have pointed out to the need to search new and better photosensitizers. As a result, several different tetrapyrrolic macrocycles, with adequate physicochemical and biological features (amphiphilicity, stability, no toxicity in the dark, red absorption), have been synthesized and evaluated as new photosensitizers in PDT; some of these are now under clinical trials [5].

We will report our recent studies leading to the synthesis of new *meso*-tetraarylporphyrin derivatives as potential photosensitizers. Part of this work was concerned with the synthesis of dimeric forms and, in such context, preliminary *in vivo* evaluations with them were also considered. Other studies were centered on the reactivity of a porphyrin macrocycle as a dienophile or a dipolarophile in cycloaddition transformations; it was expected that the products could be novel entities of the chlorin, bacteriochlorin, isobacteriochlorin and benzoporphyrin types, with prominent absorption features in relation with PDT.

Results and Discussion.

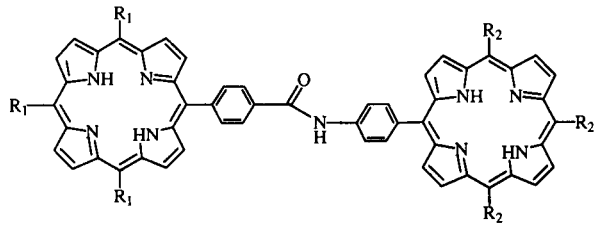
Chemistry.

Synthesis of Dimeric *meso*-Tetraarylporphyrin Derivatives *via* Amide Linkages.

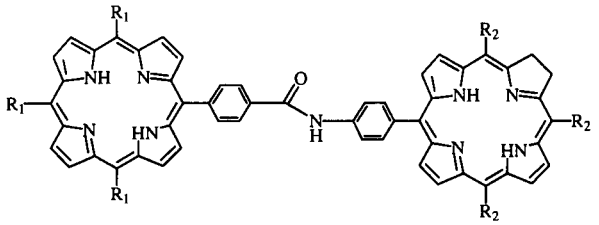
The porphyrin-porphyrin and porphyrin-chlorin dimers under study are shown in Figure 1. These compounds were obtained from monomeric intermediates [6], which are also shown in Figure 1. Coupling of the acyl chloride of porphyrin **2** with the amino-substituted compounds **4**, **6**, **8** and **9** afforded, in good yields, the dimers **D1**, **D2**, **D3** and **D4**, respectively. The amino derivatives **4**, **6** and **8** were obtained by reduction of the corresponding nitroporphyrins **3**, **5** and **7** with sodium borohydride. The chlorin **9** was prepared by reduction of porphyrin **8** with *p*-toluenesulfonohydrazide.

Porphyrins **1**, **5** and **7**, the key compounds in the synthesis of porphyrins **3**, **6** and **8**, were obtained from the Rothemund and crossed Rothemund reactions using the appropriate benzaldehydes and pyrrole, at reflux in acetic acid and nitrobenzene. Porphyrin **3** was obtained by direct nitration of compound **1** with nitric acid.

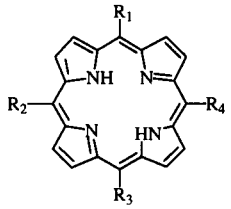
The dimers show long wavelength absorptions between λ 646 and 651 nm. The absorption results obtained for **D1**, **D2** and **D3** are typical of free-base porphyrins with the exception of the molar absorption coefficients. For these an increase by a factor of about two caused by the double absorption profile of these compounds is observed. Also for the porphyrin-chlorin dimer **D4** an increase in the long



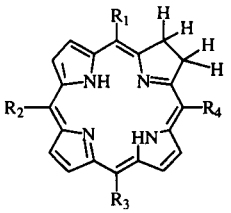
D1 $R_1 = R_2 = C_6H_5$
D2 $R_1 = C_6H_5$; $R_2 = 4\text{-}H_3COC_6H_4$
D3 $R_1 = C_6H_5$; $R_2 = 3\text{-}H_3COC_6H_4$



D4 $R_1 = C_6H_5$; $R_2 = 3\text{-}H_3COC_6H_4$



1 $R_1 = R_2 = R_3 = R_4 = C_6H_5$
2 $R_1 = R_2 = R_3 = C_6H_5$; $R_4 = 4\text{-}HO_2CC_6H_4$
3 $R_1 = R_2 = R_3 = C_6H_5$; $R_4 = 4\text{-}O_2NC_6H_4$
4 $R_1 = R_2 = R_3 = C_6H_5$; $R_4 = 4\text{-}H_2NC_6H_4$
5 $R_1 = R_2 = R_3 = 4\text{-}H_3COC_6H_4$; $R_4 = 4\text{-}O_2NC_6H_4$
6 $R_1 = R_2 = R_3 = 4\text{-}H_3COC_6H_4$; $R_4 = 4\text{-}H_2NC_6H_4$
7 $R_1 = R_2 = R_3 = 3\text{-}H_3COC_6H_4$; $R_4 = 4\text{-}O_2NC_6H_4$
8 $R_1 = R_2 = R_3 = 3\text{-}H_3COC_6H_4$; $R_4 = 4\text{-}H_2NC_6H_4$



9 $R_1 = R_2 = R_3 = 3\text{-}MeOC_6H_4$; $R_4 = 4\text{-}H_2NC_6H_4$

Figure 1. Dimers **D1-4** and porphyrins **1-8** and chlorin **9** synthesized in this work.

wavelength absorption at 651 nm, which is due to the presence of a chlorin moiety (Table 1). These features might be very significant in PDT studies since smaller doses of photosensitizers would be required.

Table 1
Absorption Properties of Dimers **D1-4** and Compound **1**

	λ_{max} (nm)	ε (mol^{-1} dm^3 cm^{-1})
D1	646	7600
D2	648	8700
D3	647	8200
D4	651	31600
Compound 1	645	3500

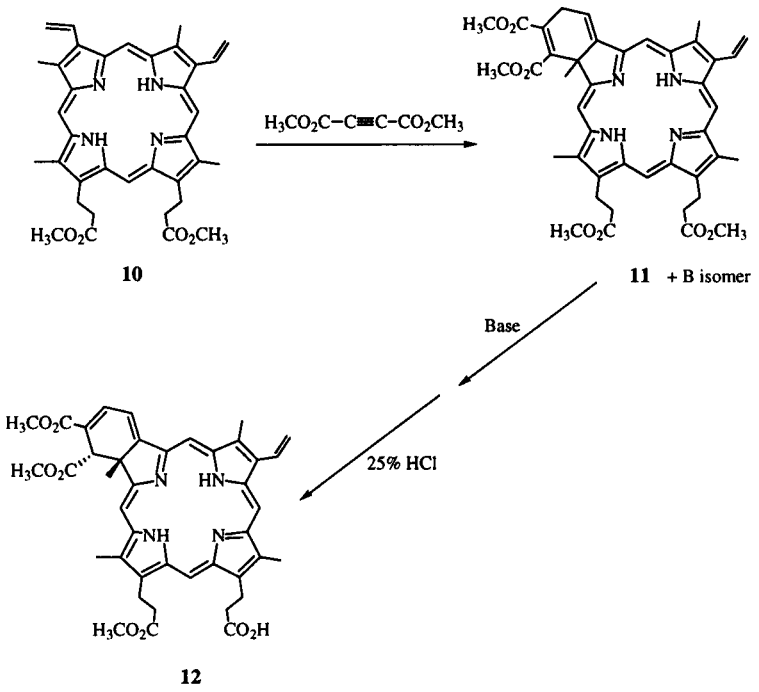
Porphyrins as Dienophiles or Dipolarophiles in Cycloaddition Transformations.

Porphyrins with vinylic moieties have been thoroughly investigated as dienes in Diels-Alder cycloadditions [7]. These porphyrins have been used as free-base macrocycles unsubstituted at the *meso*-positions, such as protoporphyrin-IX dimethyl ester **10**. In this case the formation of mono or bis-adducts was depending on the reactivity of the dienophile. Also the formation of [2 + 2] and [2 + 4] adducts has been observed. Obviously, for PDT studies the [4 + 2] adducts are promising candidates with adequate absorption features. Chlorins, bacteriochlorins and isobacteriochlorins can become available in such way: the chlorins from the reactions involving only one vinyl group, the others from the reactions of two vinyl groups located in opposite or adjacent pyrrolic units. In the case of the reaction of protoporphyrin-IX dimethyl ester with dimethyl acetylene dicarboxylate the adduct **11** is formed [8]; this adduct, upon base-catalysed rearrangement and partial acid hydrolysis, originates the benzoporphyrin derivative monoacid **12**, a compound known as BPDMA 690 which is now under PDT clinical trials (Scheme 1).

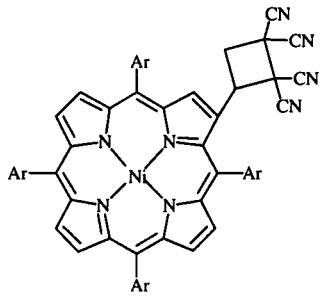
We have also demonstrated that β-vinyl-*meso*-tetraarylporphyrin nickel(II) complexes undergo [2 + 2] and [4 + 2] cycloadditions. With tetracyanoethylene as dienophile the products are porphyrins, such as **13**, and with dimethyl acetylene dicarboxylate the isolated products are the chlorin **14** and its oxidised derivative, the benzoporphyrin **15** [9]. In all these transformations the porphyrin macrocycle has acted as a diene. To our knowledge there was no reference about its use as a dienophile or a dipolarophile. We have then decided to investigate the behaviour of porphyrin macrocycles under such cycloaddition conditions. The results already obtained clearly have shown that novel products of the chlorin, bacteriochlorin, naphthoporphyrin or isobacteriochlorin types can be obtained.

meso-Tetraarylporphyrins can participate in [4 + 2] cycloadditions as the 2π electrons component with very reactive dienes, such as *o*-quinodimethanes [10]. These species are generated *in situ* by thermal extrusion of SO_2 from 1,3-dihydrobenzo[*c*]thiophene-2,2-dioxide. After heating a solution of a porphyrin **16a,b,c,d** and that sulfone in 1,2,4-trichlorobenzene, at reflux for several hours,

Scheme 1



10 **11** + B isomer **12**



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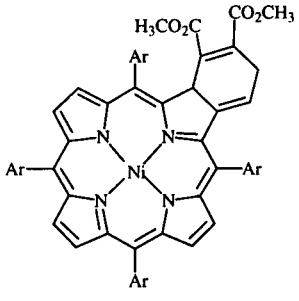

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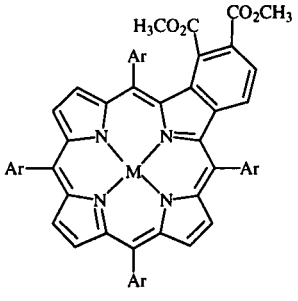

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a) M = Ni
b) M = 2 H

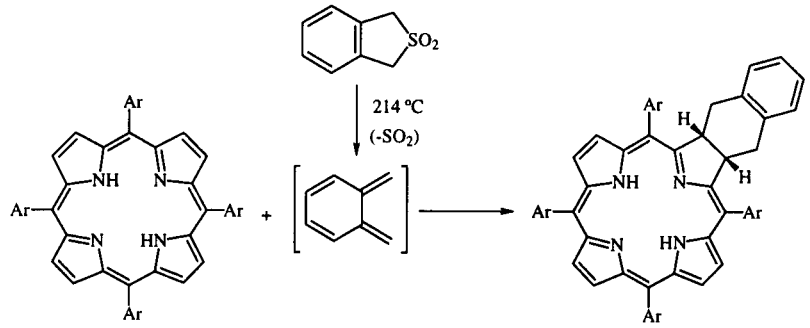
Figure 2.

the new chlorins **17a-d** could be isolated after thin layer chromatography of the reaction mixture (Scheme 2). Two other products, the naphthoporphyrins **18a,b,c** and porphyrins **19a,b,c** were always present. These porphyrins are likely intermediates in the oxidation of chlorins **17a,b,c** to naphthoporphyrins **18a,b,c** (Figure 3).

absorptions at 747 and 761 nm, and related compounds might play a significant role in future PDT processes.

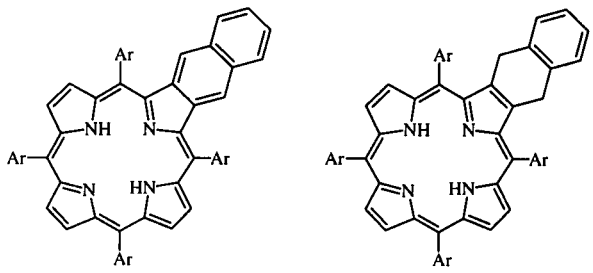
Porphyrins **16a-d** used in this work have been synthesized from pyrrole and adequate benzaldehydes (Rothemund procedure). Interestingly in the synthesis of **16d** another two minor compounds have been isolated.

Scheme 2



16 **a**, Ar = C_6H_5
b, Ar = *m*-$CH_3OC_6H_4$
c, Ar = *p*-$CH_3OC_6H_4$
d, Ar = C_6F_5

17



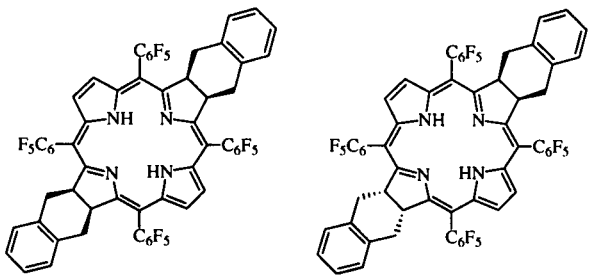
18 **a**, Ar = C_6H_5
b, Ar = *m*-$CH_3OC_6H_4$
c, Ar = *p*-$CH_3OC_6H_4$

19

Figure 3.

When *meso*-tetra(pentafluorophenyl)porphyrin **16d** was used as the dienophile, the corresponding chlorin **17d** was accompanied by two other products. The spectroscopic data obtained for them clearly showed that these new products are diastereomeric bacteriochlorins, corresponding to structures *cis*-**20** and *trans*-**20**. The formation of these 2:1 adducts is in agreement with the more "electron-deficient" features of porphyrin **16d** when compared with the other porphyrins **16a-c**. Bacteriochlorins **20**, with

Extensive analysis by mass spectrometry, nuclear magnetic resonance spectroscopy and single crystal X-ray diffraction has allowed the establishment of their structures as being the stable hexaphyrins **21** and **22** [11].

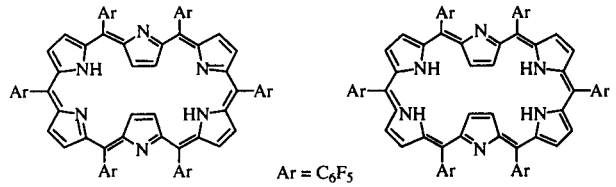


cis-**20** *trans*-**20**

Figure 4.

Porphyrins as Dipolarophiles.

The results obtained with porphyrins as dienophiles prompted us to investigate the use of such macrocycles in other cycloaddition reactions, namely the 1,3-dipolar ones.



21 22

Figure 5.

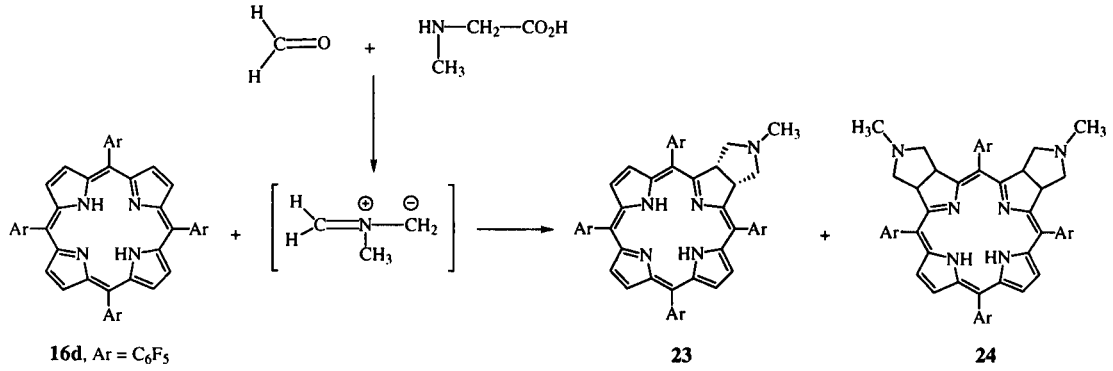
Azomethine ylides have been the 1,3-dipolar species considered so far. They were generated *in situ* by thermal decarboxylation of immonium salts obtained from condensation of α-amino acids and paraformaldehyde.

A pyrrolidine-fused chlorin **23** (λ 652 nm, Figure 6) was obtained by heating at reflux in a toluene solution of porphyrin **16d**, *N*-methylglycine and paraformaldehyde. Another by-product was obtained in low yield. Its spectroscopic data revealed that a bis-addition took place leading to a new isobacteriochlorin **24** (λ 546 and 588 nm, Figure 6), which was purified by thin layer chromatography (Scheme 3) [12].

Assuming that **24** could be a mixture of two diastereomers and aiming to increase its yield of formation, a similar reaction was carried out but using the chlorin **23** instead of porphyrin **16d**. This reaction gave rise to a mixture of products (Scheme 4), which could be separated by thin layer chromatography. Very small amounts of two diastereomeric bacteriochlorins (λ 732 nm, Figure 6) were formed. The main isolated product was identical to the isobacteriochlorin **24** isolated previously; however, another isobacteriochlorin was also isolated, although in very low yield (relative amounts 8:1). The formation of the two isomeric isobacteriochlorins and two bacteriochlorins is not surprising since in the bis-adducts the two pyrrolidine rings can be either in a "*cis*" or "*trans*" configuration. However it can be concluded that the reaction is regio and stereoselective, yielding mainly one of four possible isomers. Mainly based on the ^{19}F nuclear magnetic resonance data of **24a** and **24b**, it was possible to assign the "*trans*" configuration to the main product **24a**.

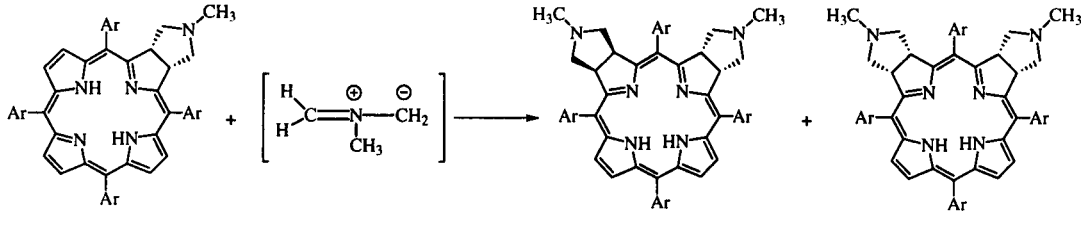
Other two porphyrins (*meso*-tetraphenylporphyrin and *meso*-tetra(2,6-dichlorophenyl)porphyrin) were used to look for the effect of the *meso*-aryl group substituents in reactions performed under the same reaction conditions. The results are presented in Table 2; it is evident that the

Scheme 3



16d, Ar = C_6F_5 **23** **24**

Scheme 4



23 **24a** **24b**

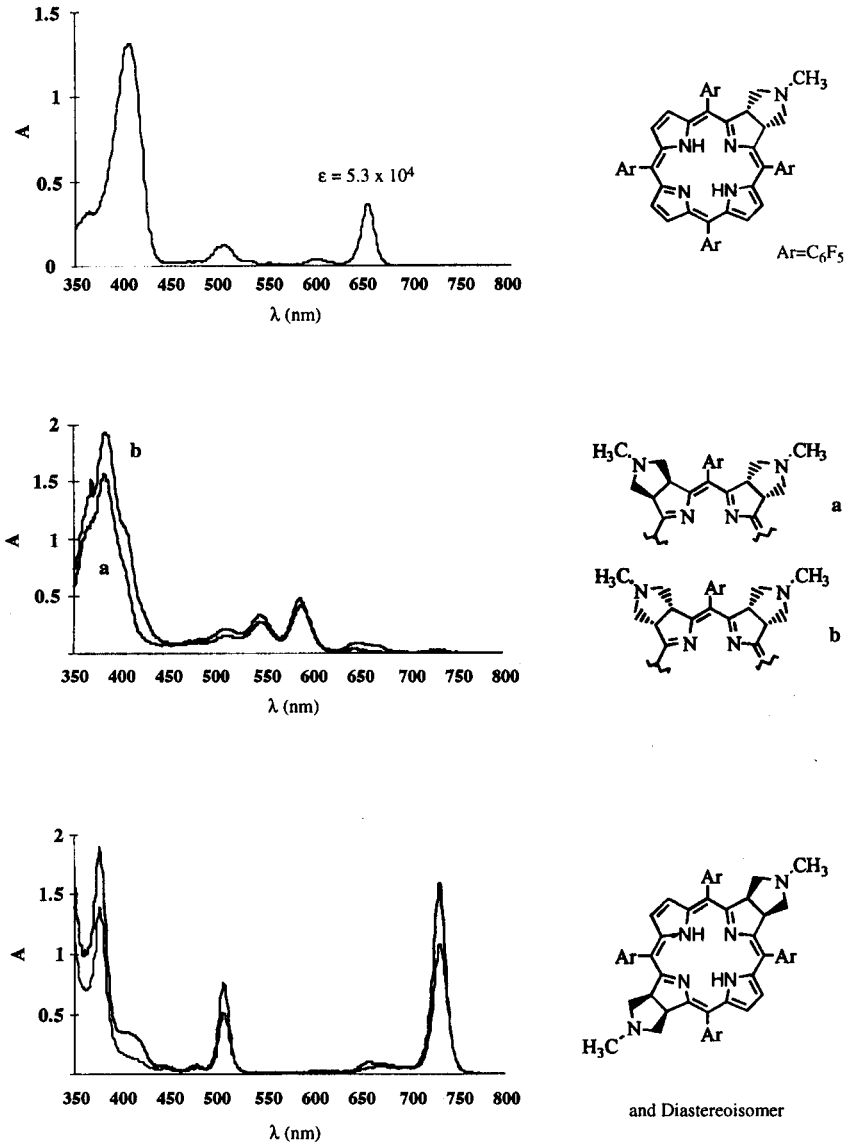


Figure 6. Visible Spectra of the New Macrocycle Derivatives.

presence of electron-withdrawing substituents in the aryl groups increases the reactivity of a porphyrin toward azomethine ylides.

Table 2
Comparative Reactivity of *meso*-Tetraarylporphyrins with Azomethine Ylides

meso-Aryl Group	Reaction Time (h)	Monoadduct (%)	Bis-adducts (%)
C_6F_5	10	61	11
2,6-$C_6H_3Cl_2$	35	26	6
C_6H_5	50	12	Not observed

Pharmacokinetic and Phototherapy Studies.

Preliminary studies were carried out with dimers **D1**, **D2**, **D3** and **D4** concerning their pharmacokinetic and photodynamic therapy properties [6]. It was also seen that the quantum yield of singlet oxygen formation by each one of the four dimers is reasonably high (0.8), which indicates that the energy transfer to oxygen is in fact the main pathway for triplet quenching.

For the *in vivo* studies female Balb/c mice were used. MS-2 Fibrosarcoma cells were suspended in sterile physiological solution and injected intramuscularly into the mice right hand leg. All pharmacokinetic and phototherapeutic studies were performed within 7-8 days after tumor implantion.

Tumor-bearing mice were intravenous-injected with each dimer in DL-α-dipalmitoylphosphatidylcholine liposomes at a dose of 1.0 or 5.0 mg/kg body weight. In the pharmacokinetic studies, and between 1 and 168 hours after administration of each photosensitizer, three mice at each time were sacrificed; blood, tumor and other tissues (liver, spleen, skin, brain, lung, kidney and muscle) were quickly processed for dimer determination. This was done with a spectrophotofluorometer (excitation at 420 nm and emission measured in the 600-750 nm range). This analytical work was carried out for each dimer; the recoveries shown in Table 3 about dimer **D3** can be considered as an example of this process. These results show that there is a good concentration ratio of each dimer for tumor/muscle and for tumor/skin at 24 hours post-injection (Table 4). This is an excellent indication for performing the PDT experiments.

Table 3
Recovery of Porphyrin Dimer **D3** from Serum and Selected Tissues of Balb/c Mice Bearing an Intramuscularly Transplanted MS-2 Fibrosarcoma at Various Times after Intravenous Injection of 1 mg/kg Photosensitizer Incorporated into DPPC Liposomes*

Post-injection Time (h)	Lung	Liver	Spleen	Kidney	Muscle	Skin	Tumor	Brain	Serum
1	1.0 ±0.3	3.3±0.3	1.8±0.1	0.7±0.1	0.1±0.0	0.3±0.0	1.1±0.1	0.1±0.0	6685.6±357.7
3	0.7±0.1	3.4±0.3	2.9±0.4	0.6±0.2	0.1±0.0	0.1±0.0	1.4±0.1	0.1±0.0	5539.1±667.8
6	0.4±0.1	6.8±0.8	3.7±0.8	0.4±0.1	0.1±0.0	0.3±0.2	2.0±0.2	nd	2550.4±392.0
15	0.2±0.0	8.1±0.5	5.2±0.8	0.2±0.1	nd	0.1±0.0	1.9±0.4	nd	115.3±13.4
24	0.1±0.0	9.5±0.5	5.1±0.7	0.1±0.1	nd	0.1±0.0	1.6±0.2	nd	41.4±6.7
48	0.1±0.0	9.0±0.4	4.3±0.4	0.1±0.0	nd	0.1±0.0	1.6±0.2	nd	4.7±1.8
168	nd	6.1±0.9	1.7±0.5	0.1±0.0	nd	0.1±0.0	0.3±0.1	nd	nd

* The recoveries are expressed as ng of drug per mg of tissue or mL of serum. The data are referred to three independently analyzed mice (average ± standard deviation); nd, not detectable.

Table 4
Dimers' Concentration Ratios Tumor/Muscle and Tumor/Skin, 24 hours after Drug Administration at a 1 mg/kg Dose

Photosensitizer	Tumor/Muscle	Tumor/Skin
D1	20	5
D2	62	24
D3	136	24
D4	4	4

In the phototherapeutic studies a group of tumor-bearing mice were injected with each dimer. Another non-injected group was kept for a later tumor growth control. Twenty four hours after the dimer administration the animals were irradiated with red light (600-700 nm) for a total light dose of 400 J cm^{-2}. The tumor volume was measured daily; its growth rate was compared with that typical of the tumors in the control mice that had not been injected or exposed to light. Growth delays of 1-1.5 days were observed with dimers **D1** and **D4** and of 2-3 days with dimers **D2** and **D3**, the latter showing a slightly better performance.

Further biological studies will be carried out with dimers **D2** and **D3** and analogues. Also with the new adducts (chlorins, bacteriochlorins and isobacteriochlorins) the determination of the photophysical parameters is taking place and, at a later stage, their photodynamic therapy features will also be undertaken.

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