Photodynamic Antitumor Agents: β-Methoxyethyl Groups Give Access to Functionalized Porphycenes and Enhance Cellular Uptake and Activity†

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Porphycene photosensitizers bearing two or four methoxyethyl side chains were synthesized in nine steps from commercially available starting materials. Ether cleavage led to (hydroxyethyl)- and (bromoethyl)porphycenes that were converted to vinyl and benzo derivatives. Five of the side chain-functionalized porphycenes were biologically studied in comparison with two tetra-*n*-propylporphycenes. Porphycenes were incorporated in small unilamellar liposomes and incubated with cultivated SSK2 murine fibrosarcoma cells. Cellular uptake and phototoxicity 24 h after 5 J/cm^2 laser light treatment were determined. The porphycenes tested were between 17 and 220 times more photodynamically active than the currently clinically used sensitizer Photofrin, although extinction coefficients of the porphycenes' irradiated bands are only approximately 10-fold higher. The LD_{50} concentration for SSK2 cells in the incubation medium was as low as $(8.5 \pm 2.8) \times 10^{-9}$ M for tetrakis(methoxyethyl)porphycene. Two methoxy or hydroxy groups enhanced cellular uptake, three or four methoxy groups both enhanced and accelerated cellular uptake of tetraalkylporphycenes. Half-life times of the uptake processes varied between (0.14 ± 0.04) and (14 ± 4) h and cellular saturation levels between (1.2 ± 0.2) and (26 ± 3) pmol/10^5 cells. When individual uptake rates were accounted for, all porphycenes had a similar "cellular" phototoxicity, pointing toward a common mechanism of action. Evidence is presented for the assumption that cell membranes are the primary targets of the tested porphycenes and that membrane solubility may play a critical role in their photodynamic efficiency. The results show that nonionic polar side chain functionalities can strongly enhance cellular uptake and antitumor activity of lipophilic porphyrinoids and thus that the known lipophilicity/activity relationship can be reversed for very hydrophobic sensitizers.

Introduction

The treatment of tumors using surgery, chemotherapy, and radiation may in the future be supplemented by certain binary techniques that develop their cytotoxic effect only during the local interplay of two otherwise nontoxic agents. Examples of these approaches that reduce systemic side effects are boron neutron capture therapy[1] and photodynamic therapy (PDT).[2] The latter induces an inactivation of cancer cells by shining red light, typically from a laser, on tumors previously loaded with injected photosensitizers.

For historical reasons, PDT trials have almost exclusively been carried out with an incompletely characterized hematoporphyrin-derived sensitizer mixture called Photofrin[3] in which the contribution of its individual components to the overall activity is not known. Therefore, in the last decade, a number of chemically pure porphyrin and porphyrinoid dyes have been synthesized and tested for their PDT efficiency.[4]

Porphycenes, porphyrin isomers initially developed purely for a scientific interest in aromatic macrocycles,[5] were found to possess high absorption above 600 nm, where tissue is relatively translucent. Soon after their first synthesis, it was observed that the porphycenes, upon irradiation, efficiently generate singlet oxygen which can induce a photodynamic effect.[6] A subsequent report of successful in vivo PDT treatment of murine tumors with a tetra-*n*-propylporphycene[7] encouraged us to explore the side chain chemistry of this sensitizer in order to optimize its properties for PDT.

We report here the synthesis and phototoxic effects on tumor cells of such side chain-functionalized porphycenes, some of which have been shown in preliminary studies to eradicate tumors in animal models[8] more efficiently than the known[9] alkyl derivatives. The results of cellular uptake studies, which help our understanding of the observed differences in photodynamic activity and in vivo pharmacokinetics[10] of these porphycenes, are included.

Results and Discussion

Synthesis. To employ the established route to 2,7,-12,17-alkylated[11] porphycenes for the synthesis of functionalized derivatives, functional groups were sought that would survive the acidic, basic, oxidative, reductive, and thermally demanding conditions of this reaction sequence[9] and still be convertable to both electrophilic and nucleophilic moieties. The stability of the porphycene macrocycle toward acidic conditions encouraged

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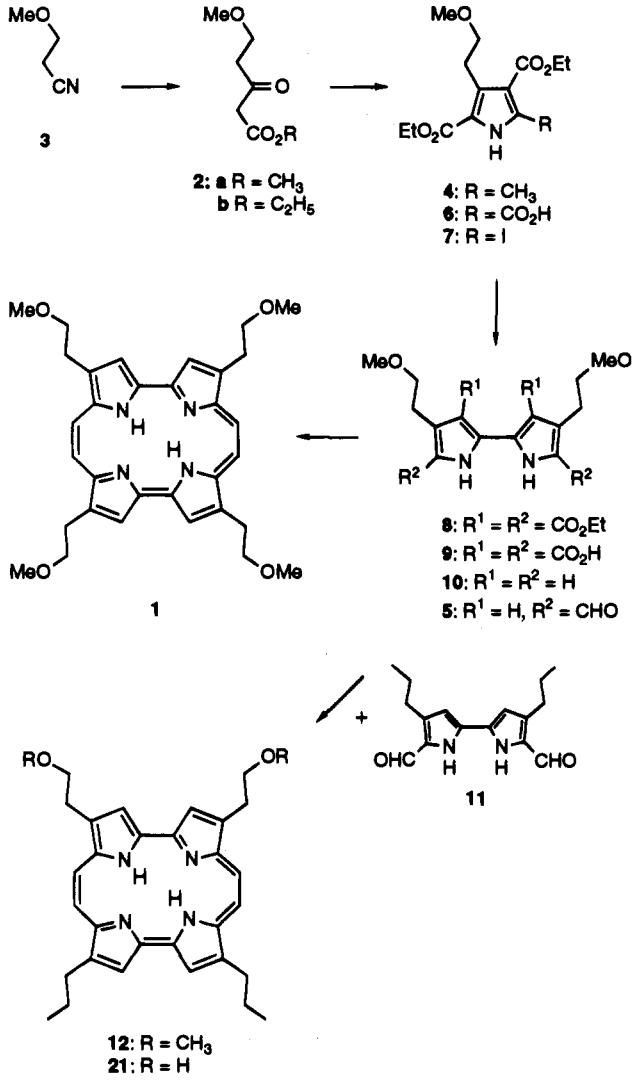
Scheme 1



us to use methoxy groups which can be demethylated with strong Lewis acids to furnish alcohols. Also, the photodynamic activity of ether porphyrins[12] and the presence of alkoxyalkyl moieties in some molecular species of the clinically used porphyrin mixture[3] suggested that these functional groups might promote the activity of PDT agents.

To gain access to the symmetrical tetrakis(β-methoxyethyl)porphycene **1**, our first target molecule (Scheme 1), β-keto ester **2a** or **2b** had to be prepared on a large scale. The three-step procedure of Wakselman[13] gave the methoxy keto ester **2a** in 29% yield. The Blaise reaction of methoxy nitrile **3** with α-bromomethyl acetate or α-bromoethyl acetate, using a zinc–copper couple,[14] furnished **2a** or **2b**, respectively, in 75% yield, after in situ hydrolysis and distillation. In a Knorr condensation, **2b** was incorporated in methylpyrrole **4** which was converted to bipyrrole dialdehyde **5** in a reaction sequence derived from classical pyrrole chemistry (ref 9 and literature cited therein). Oxidation of methylpyrrole **4** to carboxypyrrole **6** via its trihalide required careful control of reaction conditions to prevent side chain halogenation favored by the activating effect of the methoxy group. Decarboxylative iodination of **6** removed the extra carbon atom and furnished the activated α-iodopyrrole **7**, which was easily purified by crystallization from ethanol. Ullmann coupling of **7** produced bipyrrole tetraester **8**.

The reaction sequence **3** → **8** gave comparable yields when performed with methyl esters instead of the ethyl esters shown in Scheme 1, except for the pyrrole → bipyrrole coupling where 12% higher yields were obtained with methyl esters. A major disadvantage of the methyl derivatives, however, was their enhanced solubility in water which necessitated extractive workup procedures and led us to abandon their use for larger scale preparations.

Conventional hydrolysis of tetraester **8** gave the corresponding tetraacid **9** which was fully decarboxylated by sublimation at 230 °C. The α-free bipyrrole **10** showed the expected high tendency to polymerize and had to be handled with care. A final Vilsmeier formylation (**10** → **5**) set up the carbon framework for the porphycene target molecule **1**. The chosen route made dialdehyde **5** available from **4** on a gram scale in 22% overall yield without any chromatography.

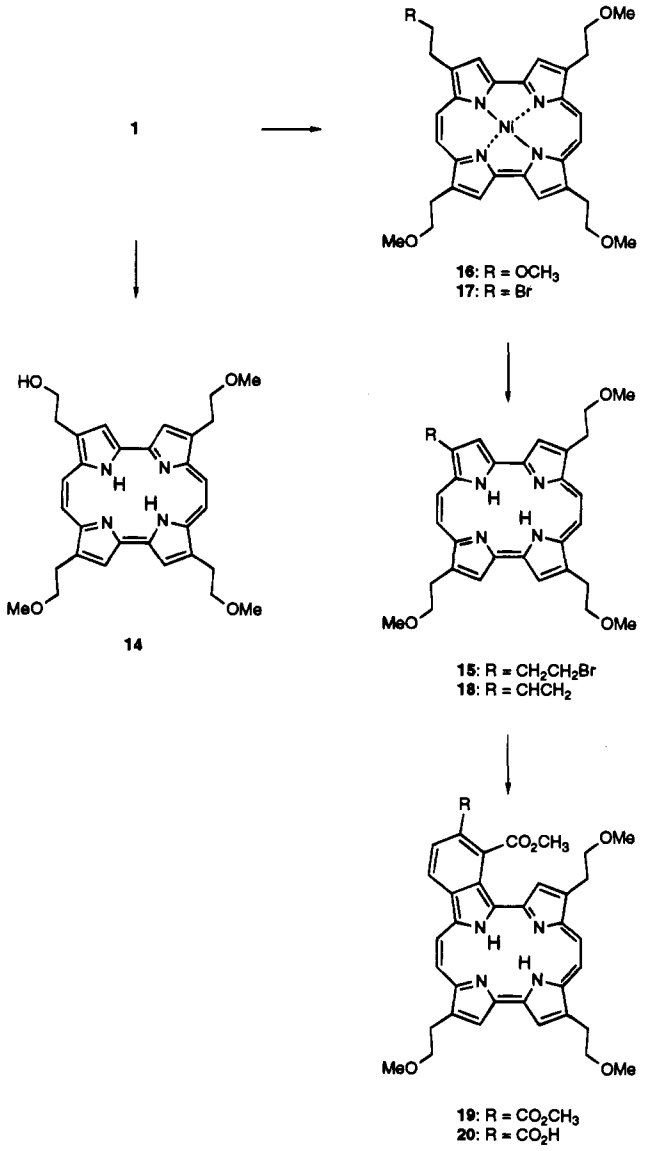
The reductive cyclization of two molecules of dialdehyde **5** was effected in a McMurry reaction with low-valent titanium in THF.[15] Tetraether porphycene **1**, probably formed via a *N,N*-dihydro intermediate, was isolated in 25% yield after chromatography and crystallization. The higher yield of the cyclization compared to that of alkylporphycenes[9] can be explained both by the higher solubility of **5** in THF and the stronger directing effects of the side chains which make the concurrent formation of polymers less dominating.

When equimolar amounts of **5** and dipropylbipyrrole dialdehyde **11**[9] were used in the McMurry coupling, porphycene diether **12** was formed in 11% yield along with tetrapropylporphycene **13** (Scheme 3) and **1** in the statistically expected 5.5% yield.

Functional group interconversion of methoxy groups was successfully performed on the porphycene level (Scheme 2). Either symmetrical (data not shown) or mono-refunctionalized derivatives were predominantly formed, depending on the amount of Lewis acid added. Treatment of tetraether porphycene 1 with boron tribromide in dichloromethane lead to (β-hydroxyethyl)porphycenes, whereas, unexpectedly, in the presence of boronic acid, the same reagent gave β-bromoethyl derivatives when a nickel atom had previously been incorporated in the porphycene cavity. Thus, under nonoptimized reaction conditions, (hydroxyethyl)porphycene **14** was generated from **1** in 18% yield (43% based on recovered starting material) and the corresponding (bromoethyl)porphycene **15** in 30% yield, via **16** and **17**. Chelation of Ni^{2+} (**1** → **16**) and demetalation with concentrated sulfuric acid (**17** → **15**) proceeded in high yield, making this sequence a valuable alternative to bromination of monoalcohol **14**.

Dehydrohalogenation of (bromoethyl)porphycene **15** with DBU furnished vinylporphycene **18** in 85% yield. The susceptibility of **18** to photooxidation, probably via [4 + 2] addition of self-sensitized singlet oxygen, required the exclusion of air and subdued light during handling. Like vinylporphyrins,[16] **18** underwent [4 + 2] Diels–Alder reactions with acetylene diesters as dienophiles. Under the cycloaddition conditions given for protoporphyrin diesters[16d] (toluene, 3–6 days of reflux), **18** mainly underwent decomposition. When **18** was directly dissolved in dimethyl acetylene dicarboxy-

Scheme 2



late (DMAD), the reaction started at 65 °C and was complete within 90 min. Under these conditions, the dihydrobenzoporphycene that was probably formed initially underwent immediate oxidative aromatization to give benzoporphycene dimethyl ester **19** which was isolated in 60% yield after chromatography. Reports on the high photodynamic activity of hydrobenzoporphyrin monoacids[17] led us to perform a careful hydrolysis of benzo diester **19** with LiOH in THF/water. After 5 h at room temperature, 65% of the desired monoacid **20** was formed, whose structure was elucidated by two-dimensional NMR experiments (COSY and $^{13}C-^{1}H$ correlation spectroscopy). The preferential cleavage of the "outer" ester group was probably due to the stronger steric shielding of the second methoxycarbonyl moiety.

A symmetrical bis(hydroxyethyl)porphycene, **21** (Scheme 1), was generated from diether porphycene **12** in 70% yield with the chemistry established for the monoalcohol **14**. In a different approach to porphycene functionalization, tetrapropylporphycene **13** was converted to its 9-acetoxy derivative **22** (Scheme 3).[18]

All new functionalized derivatives retained typical spectroscopic characteristics of porphycenes and metalloporphycenes (see experimental part). Only some

Scheme 3

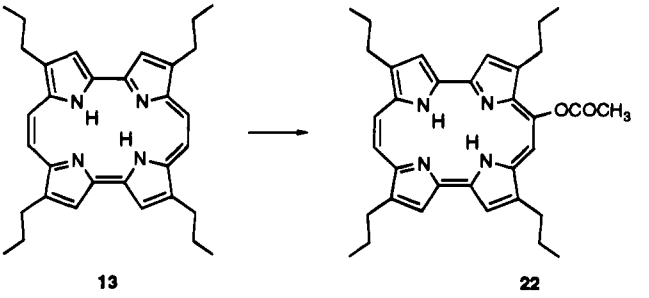


Table 1. UV/Vis Maxima of Porphycene and Substituted Porphycenes in CH_2Cl_2

	λ_{max}[nm] (ϵ[M^{-1} cm^{-1}])	
sensitizer	B-bands	Q-bands
porphycene[a]	358 (139 200)	558 (34 200), 596 (30 400), 630 (51 900)
1	370 (140 000)	563 (35 000), 602 (33 000), 634 (47 000)
12	370 (142 000)	562 (36 000), 601 (34 000), 633 (49 000)
13[b]	370 (142 000)	560 (36 400), 600 (34 100), 633 (48 200)
14	370 (134 000)	563 (34 000), 602 (31 000), 635 (44 000)
15	370 (133 000)	564 (34 000), 603 (32 000), 635 (45 000)
16	387 (127 000)	603 (63 000)
17	387 (128 000)	605 (65 000)
18	373 (98 0000)	572 (26 000), 613 (28 000), 643 (33 000)
19	396 (101 000)	576 (32 000), 612 (60 000), 639 (42 000)
20[c]	396 (89 000)	576 (27 000), 610 (55 000), 636 (36 000)
21	369 (138 000)	562 (36 000), 601 (34 000), 633 (48 000)
22	372 (143 000)	562 (30 000), 603 (33 000), 632 (32 000), 641 (31 000)

[a] From ref 5. [b] From ref 9. [c] in $CHCL_3$/MeOH (9:1).

data relevant to the present study will be briefly mentioned, and the reader is kindly referred to the literature for more detailed investigations.[5,6,9,19] Proton NMR spectra of the new porphycenes showed perimeter protons at low field (9–10 ppm) and NH resonances at high field (0–3.5 ppm) due to the strong diamagnetic ring current of the aromatic macrocycle. UV/vis absorption bands relevant to the in vivo sensitization were only slightly changed by the presence of β-ethyl substituents compared to unsubstituted porphycene (Table 1). The vinyl group in **18** induced the expected moderate rhodifying effect.[20] Acetoxyporphycene **22** and benzoporphycenes **19** and **20** all showed a slight bathochromic shift of all bands with an increased absorption of the second Q-band in **19** and **20** and a splitting of the longest wavelength band in **22** (Figure 1). The benzoporphycenes also showed a red shift of the B-bands toward a wavelength more typical for porphyrins.[20] Nickel complexes **16** and **17** had spectral characteristics similar to the tetrapropyl derivative described previously.[9] The strong red fluorescence (Φ_f within 0.3 ± 0.1[21]) of all but the nickel porphycenes, together with intense and well-separated B- and Q-bands, allowed a selective and highly sensitive detection in cells (see biological part) and might make these derivatives potential candidates for early stage cancer diagnosis.[22]

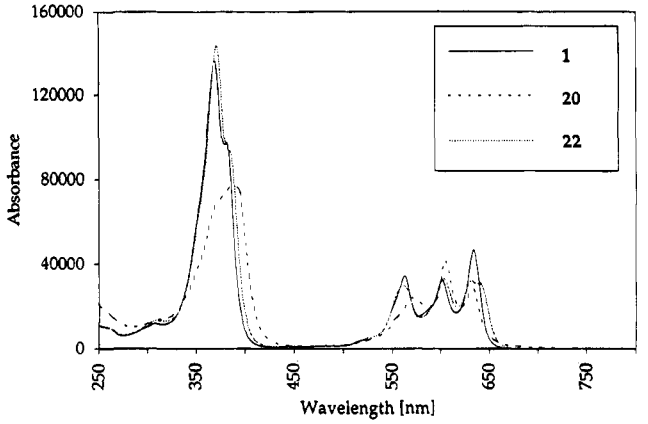


Figure 1. UV/vis spectra of **1** and **22** in CH_2Cl_2 and **20** in $CHCl_3$/MeOH.

Table 2. Photodynamic Activity of Sensitizers[a]

sensitizer	LD_{50} [nM]	ϵ-corr LD_{50} [nM]
Photofrin	1900 ± 300	
alkylporphycenes		
13	77 ± 7	77 ± 7
22	110 ± 20	73 ± 13
side chain functionalized porphycenes		
21	12 ± 4	12 ± 4
1	8.5 ± 2.8	8.3 ± 2.7
14	9.1 ± 2.1	8.3 ± 1.9
20	13 ± 3.0	9.7 ± 2.2

[a] LD_{50} concentrations in incubation media (±limits of the 95% confidentiality interval) as obtained by probit analysis of SSK3 fibrosarcoma cell viability, determined by trypan blue exclusion, 24 h after 5 J/cm² irradiation. ϵ-corr values are corrected for extinction coefficients of the irradiated maxima (Table 1), relative to **13**.

Porphycenes **1**, **12**–**14**, and **20**–**22**, chosen for biological testing, all possessed octanol/water partition coefficients >250, and monoacid **21** only became noticeably water soluble upon basification and addition of methanol. Thus all these porphycenes are strongly lipophilic molecules.

Biological Experiments. The phototoxic effect of porphycenes on SSK2 cultivated murine fibrosarcoma cells was measured in comparison to that of Photofrin. For the incubation with cells, porphycenes were incorporated in known[7] and photophysically studied[23] DPPC liposomes. At any given concentration, cell viability, determined by dye exclusion 24 h after 5 J/cm² laser light irradiation, was considerably lower with porphycenes than with Photofrin, but cell survival curves had a similar shape (Table 2, Figure 2). Among the porphycenes tested, a clear-cut difference in activity was observed between side chain-functionalized and alkyl derivatives. Cell inactivation, expressed as LD_{50} of a probit analysis,[24] required approximately $^1/_{20}$ of the Photofrin concentration in the incubation medium for alkylporphycenes and approximately $^1/_{200}$ of this concentration for β-oxoporphycenes. Activity differences within the two groups were the same within experimental error when the individual extinction coefficients were taken into account (Table 2). Thus, the photodynamic efficiency of the porphycenes did not noticeably change when a methoxyethyl group in **1** was replaced by a hydroxyethyl group (**14**) or a benzo group bearing an ester and acid group (**20**). Neither was the activity

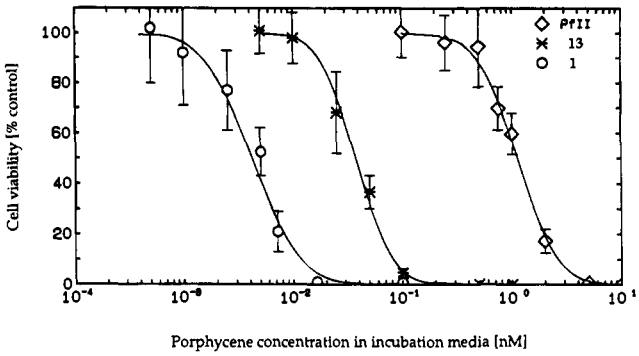


Figure 2. Viability of fibrosarcoma cells, determined by trypan blue exclusion, 24 h after incubation and 5 J/cm² laser light irradiation, for porphycenes **1**, **13**, and Photofrin (Pf II). Values are the mean of at least four independent experiments ±SD; lines represent results of probit analysis.

of **13** strongly influenced by the ester group in **21** attached to the porphycene nucleus. Change from perimeter to side chain functionalization did, however, enhance photodynamic potency by 1 order of magnitude.

The strong increase in photodynamic activity of porphycenes induced by the polar oxo substituents was unexpected in terms of the known structure–activity relationships for porphyrinoid sensitizers. Kessel and Chou[25] have shown that lipophilicity *increases* the photosensitizing capacity of porphyrins, a finding that was later extended by Moan et al. to other porphyrinoids.[26] Van Lier's group demonstrated that among isomers of sulfonated phthalocyanines the amphiphilic molecules were the most potent sensitizers,[27] which was confirmed by others.[28] The photodynamic efficiency of porphycenes *decreased*, however, with lipophilicity, and this effect was *not* induced by amphiphilicity as it also occurred for symmetrically substituted derivatives, e.g., **1** and **13**. In fact, the most amphiphilic molecule tested, carboxyporphycene **20**, was a slightly *less* effective sensitizer than the symmetrical parent compound **1** (Table 2).

Since the photophysical characteristics of all porphycenes tested were similar,[21] we assumed that differences in cell concentrations or intracellular locations were responsible for the observed effect, rather than different sensitizer efficiencies. Accordingly, the uptake of selected porphycenes in fibrosarcoma cells was studied by flow cytometry under the same incubation conditions and using the same liposome formulation as in the phototoxicity assay (Figure 3). In this experiment, the uptake kinetics of the sensitizers fell into the same two groupings observed in the photodynamic activity experiments. Porphycene fluorescence rose slowly and steadily with incubation time for the alkylporphycenes **13** and **22**, whereas ether porphycenes **1** and **14** reached fluorescence maxima within 1 h. The ether porphycenes also stained cells far more intensely than the derivatives bearing hydrocarbon side chains.

To correlate the observed fluorescence with sensitizer concentrations, cellular porphycene levels were determined by extraction (Figure 4, Table 3). In the experimental protocol, newly developed long-time stable DOPC liposomes were employed.[29] These were as efficient in porphycene delivery as the DPPC preparations in control experiments (data not shown). Porphycene levels in cells increased in the order alkylporphycenes < ether porphycenes, as in the fluorescence assay.

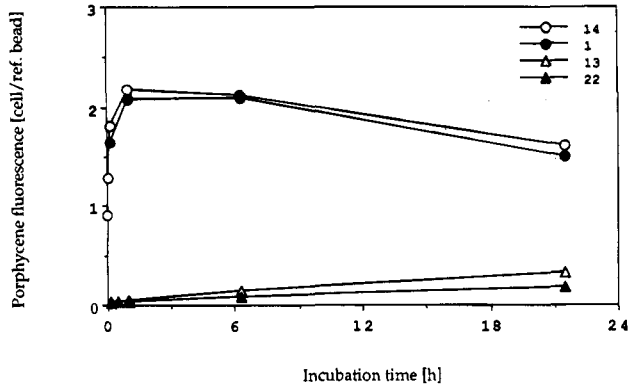


Figure 3. Porphycene fluorescence in SSK2 fibrosarcoma cells, determined by flow cytometry: excitation at 360 nm; emission >610 nm; fluorescence intensities relative to internal standard fluorescence beads.

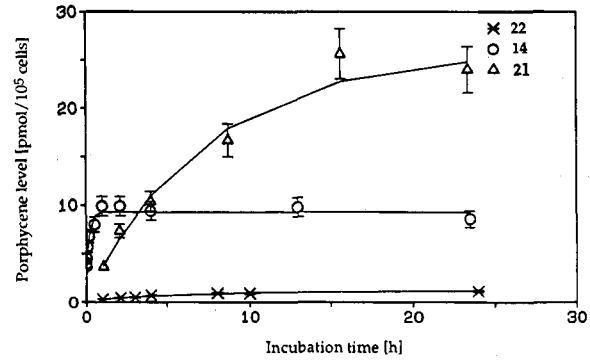


Figure 4. Cell uptake of porphycenes **14**, **21**, and **22** in SSK3 fibrosarcoma cells as determined by chemical extraction. Values are the mean of at least four independent experiments ±SD; lines represent results of probit analysis.

Table 3. Cellular Uptake Kinetics and Corrected LD_{50} Values of Porphycenes[a]

sensitizer	$t_{1/2}$ [h]	c_s [pmol/10^5 cells]	ϵ/uptake corr LD_{50}[b]
	Alkylporphycenes		
13	4.4 ± 1.6	1.5 ± 0.2	6.1
22	4.3 ± 1.2	1.2 ± 0.2	5.6
	Half Side-Chain-Functionalized Porphycenes		
12	14 ± 4	14 ± 4	
21	5.2 ± 1.3	26 ± 3	13
	Full Side-Chain-Functionalized Porphycenes		
1	[c]	[c]	8.3
14	0.14 ± 0.04	9.3 ± 0.4	8.3
20	0.55 ± 0.1	7.3 ± 0.4	7.2

[a] Results of monoexponential fits to porphycene uptake in SSK3 fibrosarcoma cells as determined by chemical extraction. $t_{1/2}$ (±SD) denotes times to reach half-saturation; c_s are saturation cell levels. LD_{50} values are porphycene concentrations, as specified in Table 2, corrected for differences of extinction coefficients and uptake rates, after 4 h relative to **14**, which was known to be taken up to saturation (see Figure 4). [b] Standard error not determinable. [c] Estimated to equal **14** (see Figure 3).

Under saturation conditions, the uptake enhancement (c_s) over alkylporphycenes was 5-8-fold for triethers **14** and **20** and 9-22-fold for difunctionalized porphycenes **12** and **21**. Time constants of cellular uptake (Table 3) divided the group of side chain-functionalized porphycenes into "half-side" (**12**, **21**) and "fully" functionalized derivatives (**14**, **20**). The latter group was taken up in less than 1 hour, whereas the dioxo ethylporphycenes took as long (**21**) or even longer (**12**) than the alkylporphycenes to reach half of their respective plateau levels. The acceleration amounts to 1 order of magnitude in time constants and is clearly distinguishable from differences within each group. The kinetic experiments show that it takes two peripheral ether or hydroxy groups to strongly enhance the cellular uptake of alkylporphycenes but it takes four such substituents to enhance *and* accelerate them.

The cellular uptake kinetics of the sensitizers helped to explain the different LD_{50} concentrations in the phototoxicity study of the photophysically very similar porphycenes: alkylporphycenes with their slower uptake rate to a lower maximum required higher external concentrations to reach cellular LD_{50} levels than their functionalized counterparts. The quantitative validity of this reasoning can be seen from the extinction/uptake-corrected LD_{50} concentrations in Table 3. For this correction, a factor had been derived from the cellular uptake rates of individual porphycenes within the incubation time of the phototoxicity experiment (4 h). The thus calculated "cellular LD_{50} values" differ by no more than 2, with better correlation within subgroups. Exclusion of the non-ether-functionalized derivative **21** even yields a numerical agreement within the experimental errors of the two experiments.

This comparable "cellular" photodynamic activity of the different porphycenes implies that the photosensitization occurs at subcellular structures of similar sensitivity. The reported damage to membranes by certain other porphyrinoid photosensitizers[30] and several lines of evidence lead us to the tentative conclusion that cell membranes are the cellular structures that are the primary targets of porphycene sensitization. A pronounced cell swelling was observed by microscopy in SSK2 cells that had been sensitized with porphycenes and irradiated, in accordance with an impairment of cell membrane function. Fluorescence images of cells incubated with both alkylporphycenes and ether porphycenes predominantly showed cell membrane-associated fluorescence (data not shown). Subsequent to our results, it was found by Wessels and Nüsse in a flow cytometric investigation that the fast penetrating ether porphycene **14** can even be used to discriminate micronuclei from cell debris due to a staining of their membrane.[31]

Furthermore, the solubility of several porphycenes in liposomal membranes[29] roughly correlates inversely with their LD_{50} concentrations in the cellular phototoxicity experiment (Table 4). Compared to the alkylporphycenes **13** and **22**, both ether porphycenes (**14**, **20**) are approximately 1 order of magnitude more soluble in the membrane vesicles and are accordingly photodynamically effective at lower concentrations. The fact that the available qualitative[8a] and quantitative[8b] (Table 4, third column) data on in vivo photodynamic efficiency of porphycenes show the same graduation indicates the relevance of this membrane sensitization proposal. Additionally, when intravenously injected into hamsters, porphycenes **14** and **20** have shown distinctly accelerated serum kinetics and erythrocyte uptake compared to **22** and Photofrin,[10] demonstrating that the cellular kinetic study herein also has validity for the in vivo situation. The first measurements of diffusion coefficients in tissue further complement this picture.[32]

It is noteworthy that the less well-distributing alkylporphycene **22** has shown a much more selective

Table 4. Comparison of Liposomal Solubilities and in Vitro and in Vivo Phototoxic Doses of Photosensitizers

sensitizer	liposome solubility[a] [ratio sens/lipid for 95% incorporation]	LD_{50} for cultivated SSK2 cells[b] [μM]	dose for 1 w regrowth delay of transplanted SSK2 tumors in C3H mice[c] [mg (μmmol)/kg bw]
Photofrin		19 ± 3	20 (33)
alkylporphycenes			
13	1:200	0.8 ± 0.1	
21	1:350	1.1 ± 0.2	2 (3.7)
side chain-functionalized porphycenes			
14	1:45	0.09 ± 0.02	0.5 (0.95)
20	1:35	0.13 ± 0.03	

[a] Estimated error ±15%, from ref 29. [b] Concentrations in incubation media as determined 24 h after 5 J/cm^2 irradiation; see Table 2 for details. [c] iv injected doses, prior to 200 J/cm^2 irradiation, from ref 8b.

macroscopic tumor accumulation than ether porphycenes **14** and **20** but is less therapeutically effective than these ether porphycenes which also do not induce intolerable side effects at their required lower dosages. Alkylporphycene **22** probably remains predominantly within the interstitial fluid of the neoplasms[10] and thus might not reach the more sensitive cell membranes. We conclude that a consideration of macroscopic and microscopic tumor targeting might help to develop further optimized porphyrin(oid)s for both tumor diagnostics and photodynamic therapy.

Conclusions

(Methoxyethyl)porphycenes are accessible from commercially available acyclic starting materials and can be converted to hydroxyethyl, bromoethyl, vinyl, and benzo derivatives on the porphycene level. Two methoxy groups were found to enhance and four methoxy groups were found to both enhance and accelerate the uptake of porphycenes, resulting in sensitizers with activities 2 orders of magnitude above the activity of the routinely used porphyrin mixture Photofrin. The conversion of one of these methoxy groups to a hydroxy group or an ester carboxy benzo substituent or two ether groups to hydroxyl groups did not strongly affect their beneficial effect on photodynamic efficiency of the porphycene derivatives.

Our results indicate that a maximum photodynamic efficiency of porphycenes occurs at a solubility below the lipophilicity determinable by "classical" octanol/water partition coefficients and above the rigorous lipophilicity of the aromatic macrocycle bearing only hydrocarbon side chains. For similar porphyrinoids, designed to operate in membranes, the determination of their membrane solubility, e.g., via liposome incorporation efficiency,[29] can possibly be useful for the prediction of their photodynamic activity. The effort to establish such abiotic tests might prove worthwhile, taking into account that the in vitro activities reported here correlate with therapeutic responses in animal tumor models.

Experimental Section

Synthesis of Porphycenes. Nuclear magnetic resonance spectra were obtained on a Bruker AM 300 or a Bruker AC 80 spectrometer, and chemical shifts are expressed in parts per million downfield from tetramethylsilane. UV/visible spectra were recorded on a Perkin-Elmer Lambda 7 spectrophotometer; absorptions are given in nanometers and extinction coefficients in M^{-1} cm^{-1}. Mass spectra were measured on Finnigan mass spectrometers, Models 3200, MAT 212, and MAT H-SQ 30. Infrared spectra were acquired on a Perkin-Elmer IR spectrophotometer 283. Analytical TLC was performed by using Machery-Nagel (Düren, Germany) silica gel SIL G/UV 254 precoated sheets. Column chromatography was performed by using Merck (Darmstadt, Germany) silica gel 60, or aluminum oxide 90 (both 0.063–0.2 mm). Elemental analyses were performed by the Analytical Department of Bayer AG, Leverkusen.

THF and CH_2Cl_2 used for reactions were freshly distilled over $LiAlH_4$ in an argon atmosphere; all solvents and reagents were at least analytical grade.

5-Methoxy-3-oxopentanoic Acid Alkyl Esters 2a,b. 3-Methoxypropionitrile (**3**) (68 g, 0.8 mol) was dissolved in 1 L of THF and treated with 150.3 g (2.2 mol) of Zn/Cu pair[14] under argon. Under moderate reflux, 267.2 g (1.6 mol) of ethyl bromoacetate was added to the vigorously stirred suspension over 75 min. The solution was stirred for an additional 30 min at reflux. Then, 420 mL of 10% HCl was added within 30 min at 15 °C and the solution stirred for another 30 min. The solution was filtered, and the filtrate was extracted three times with $CHCl_3$. The organic phases were washed four times with water and once with 5% $NaHCO_3$ and dried, and the solvent was removed in vacuo. The resulting reddish liquid was distilled. The fraction at 85 °C/0.5 Torr yielded 105 g (0.6 mol) of 5-methoxy-3-oxopentanoic acid ethyl ester (**2b**) as a colorless, fruity smelling oil with a refractive index of n^{22}_D = 1.434. Yield: 75% based on **3**. ^1H NMR ($CDCl_3$, 80 MHz): δ 1.06 (t, 3H, CH_2CH_3), 2.57 (t, 2H, $MeOCH_2CH_2$), 3.10 (s, 3H, CH_3OCH_2), 3.28 (s, 2H, $COCH_2CO$), 3.43 (t, 2H, $MeOCH_2$), 3.97 (q, 2H, CH_2CH_3). MS (EI, 70 eV): (*m/e*, rel intensity) 174 (M^+, 1), 143 ($M - OMe^+$, 12), 100 ($M - MeOCH_2 - CH_2CH_3^+$, 40), 87 ($M - CH_2CO_2Et^+$, 85). Anal. ($C_8H_{14}O_4$) C,H.

If *methyl* bromoacetate was added as the 2-bromo ester, 97.2 g (0.61 mol) of 5-methoxy-3-oxopentanoic acid methyl ester (**2a**) having a boiling point of 62–63 °C/0.12 Torr was obtained with the same method. The refractive index of the colorless oil, produced in 76% yield based on **3**, was n^{21}_D = 1.435. ^1H NMR ($CDCl_3$, 80 MHz): δ 2.62 (t, 2H, $MeOCH_2CH_2$), 3.16 (s, 3H, CH_3OCH_2), 3.35 (s, 2H, $COCH_2CO$), 3.48 (t, 2H, $MeOCH_2$), 3.56 (s, 3H, CO_2CH_3), signals at 4.9 (s) and 2.3 (t) through the enol form present in the equilibrium. MS (EI, 70 eV): (*m/e*, rel intensity) 160 (M^+, 3), 129 ($M - OMe^+$, 12), 101 ($M - CO_2Me^+$, 40), 100 ($M - MeOCH_2 - CH_3^+$, 33), 87 ($M - CH_2CO_2Me^+$, 85).

2,4-Bis(ethoxycarbonyl)-3-(methoxyethyl)-5-methylpyrrole (4). A cold saturated aqueous solution of 42 g (0.6 mol) of sodium nitrite was added so slowly to a stirred, ice-cooled solution of 104.4 g (0.6 mol) of **2b** in 450 mL of glacial acetic acid that no NO_x development was detected. After the solution was stirred for 2 h at room temperature, 83.2 g (0.64 mol) of acetoacetic acid ethyl ester was added. Under vigorous stirring, a mixture of 80 g (1.22 mol) of zinc powder and 100 g (1.22 mol) of sodium acetate was added over 40 min while the temperature was kept at 80–85 °C by occasional cooling. After an additional 30 min, the solution was filtered, poured into 4 L of water, and left standing overnight. The precipitate was filtered off, washed with a little cold ether, and dried over P_4O_{10} in vacuo overnight. For the subsequent transformation of **4** → **6**, crystallization was not necessary. However, when crystallized from ethanol/water, 113.2 g (0.4 mol) of **4** was obtained as colorless needles. Mp: 85 °C. Yield: 65% based

on **2b**. ^{1}H NMR ($CDCl_3$, 80 MHz): δ 1.33 (2t, 6H, CH_2CH_3), 2.48 (s, 3H, α-CH_3), 3.35 (s, 3H, CH_3OCH_2), 3.45 (A_2B_2, 4H, $MeOCH_2CH_2$), 4.27 (q, 2H, β-$CO_2CH_2CH_3$), 4.32 (q, 2H, α-$CO_2CH_2CH_3$), 9.5 (br s, 1H, NH). UV/vis (CH_2Cl_2): λ_{max} 226 (13 000), 271 (16 000). Anal. ($C_{14}H_{21}NO_5$) C, H, N.

2-Carboxy-3,5-bis(ethoxycarbonyl)-4-(methoxyethyl)-pyrrole (6). Distilled bromine (48 g, 0.3 mol) was added at once to a solution of 85 g (0.3 mol) of α-methylpyrrole diethyl ester **4** in 280 mL of glacial acetic acid and 55 mL of acetic anhydride at 0 °C; 128.5 g (1.05 mol) of freshly distilled sulfuryl chloride was added at this temperature in the dark over 2 h. The resulting red viscous solution obtained was stirred at 0 °C for 2 h. Then, 190 mL of water was added over 30 min, and the inner temperature rose to 70 °C. After 30 min of additional stirring, the mixture was poured into 2.5 L of water. The suspension was left standing for 12 h and filtered. The residue was suspended in 2 L of water at 70 °C and treated in portions with solid $NaHCO_3$ until generation of CO_2 was no longer observed. The mixture was filtered and slowly neutralized with semiconcentrated HCl while stirring vigorously. The mixture was kept at 0 °C for 8 h, and the resulting fine crystalline solid was filtered off. **6** was recrystallized from ethanol/water and dried under vacuum for 2 days; 47 g (0.15 mol) of colorless needless melting at 136 °C was obtained. Yield: 50%. ^{1}H NMR ($CDCl_3$, 80 MHz): δ 1.38 (t, 3H, β-$CO_2CH_2CH_3$), 1.45 (t, 3H, α-$CO_2CH_2CH_3$), 3.33 (s, 3H, CH_3OCH_2), 3.46 (A_2B_2, 4H, $MeOCH_2CH_2$), 4.40 (q, 2H, β-$CO_2CH_2CH_3$), 4.50 (q, 2H, α-$CO_2CH_2CH_3$), 10.25 (br s, 1H, NH), 14.64 (br s, 1H, CO_2H). MS (EI, 70 eV): (*m/e*, rel intensity) 313 (M^+, 34), 281 ($M - MeOH^+$, 41), 252 ($M - EtOH - CH_3^+$, 28), 222 ($M - MeOCH_2 - EtOH^+$, 48), UV/vis (CH_2Cl_2): λ_{max} 237 (27 000), 257 (9200), 288 (8000). Anal. ($C_{14}H_{19}NO_7$) H,N; C: calcd, 53.67; found, 53.10.

3,5-Bis(ethoxycarbonyl)-2-iodo-4-(methoxyethyl)pyrrole (7). While stirring, 47 g (0.15 mol) of pyrrolecarboxylic acid **6** was suspended in 350 mL of water and treated in portions with 40.9 g (0.48 mol) of $NaHCO_3$ at 75 °C to form a clear solution; 38.1 g (0.15 mol) of I_2 and 49.8 g (0.3 mol) of KI in 280 mL of water were added dropwise over 2 h, during which CO_2 formation was observed. **7** began to precipitate after 15 min. After completion of the addition, the mixture was heated to 75 °C for 30 min and the warm reaction solution was then poured onto 700 g of ice. The precipitate was filtered off, washed with water and ice cold pentane, and recrystallized from ethanol to give colorless needles; 53.3 g (0.135 mol) of **7** was obtained. Mp 132 °C. Yield: 90%. ^{1}H NMR ($CDCl_3$, 80 MHz): δ 1.36 (t, 3H, β-$CO_2CH_2CH_3$), 1.37 (t, 3H, α-$CO_2CH_2CH_3$), 3.34 (s, 3H, CH_3OCH_2), 3.46 (A_2B_2, 4H, $MeOCH_2CH_2$), 4.33 (q, 2H, β-$CO_2CH_2CH_3$), 4.36 (q, 2H, α-$CO_2CH_2CH_3$), 9.5 (br s, 1H, NH). MS (EI, 75 eV): (*m/e*, rel intensity) 395 (M^+, 30), 363 ($M - MeOH^+$, 100), 322 ($M - CO_2Et^+$, 31), 276 ($M - EtOH - C_2H_5 - MeOCH_2^+$, 42). UV/vis ($CH_2Cl_2$): λ_{max} 227 (14 000), 267 (18 000). Anal. ($C_{13}H_{18}NO_5I$) C,H,N.

3,3′,5,5′-Tetrakis(ethoxycarbonyl)-4,4′-bis(methoxyethyl)-2,2′-bipyrrole (8). Copper bronze (45 g, 0.7 mol) was added to a solution of 47.4 g (0.12 mol) of iodopyrrole diethyl ester **7** in 250 mL of absolute DMI, and the mixture was stirred at room temperature for 20 h. The suspension assumed a greenish-brown color. The crude product was precipitated by slowly adding 1.2 L of water and filtering off over Celite together with the nonconverted copper. The filter residue was extracted with 400 mL of hot chloroform. The extract was washed briefly with 20% HNO_3, twice with water, and once with 5% $NaHCO_3$. The solvent was evaporated after drying over $MgSO_4$ and the remaining solid washed with 50 mL of cold hexane followed by high-vacuum drying; 21.5 g (40.2 mmol) of **8** was crystallized as needles from the ethanol. Mp 150 °C. Yield: 67%. ^{1}H NMR ($CDCl_3$, 80 MHz): δ 1.41 (t, 6H, β-$CO_2CH_2CH_3$), 1.42 (t, 6H, α-$CO_2CH_2CH_3$), 3.34 (s, 6H, H_3COCH_2), 3.49 (A_2B_2, 8H, $MeOCH_2CH_2$), 4.39 (q, 4H, β-$CO_2CH_2CH_3$), 4.43 (q, 4H, α-$CO_2CH_2CH_3$), 14.10 (br s, 2H, NH). MS (EI, 75 eV): (*m/e*, rel intensity) 536 (M^+, 100), 504 ($M - MeOH^+$, 3), 490 ($M - EtOH^+$, 11), 445 ($M - MeOCH_2 - EtOH^+$, 26). UV/vis (CH_2Cl_2): λ_{max} 228 (19 000), 249 (26 000), 289 (15 000), 347 (23 000). Anal. ($C_{26}H_{36}N_2O_{10}$) C,H,N.

3,3′,5,5′-Tetracarboxy-4,4′-bis(methoxyethyl)-2,2′-bipyrrole (9). A solution of 26.8 g (50 mmol) of bipyrrole tetraethyl ester **8** in 1.35 L of methanol was treated with 30 g (0.75 mol) of NaOH in 540 mL of water and heated to reflux for 40 h. Following removal of the methanol by distillation, the mixture was diluted with water to 4 L and neutralized with HCl while stirring. The mixture was stirred at the neutral point for 15 min at 25 °C and then fully precipitated at pH 2. The suspension was left standing at 0 °C for 6 h, and the precipitate was filtered off, washed with water, and dried for 5 days over P_4O_{10} under vacuum; 19.5 g (46 mmol) of **9** was obtained as a colorless to slightly greenish powder. Recrystallization from DMSO/water yielded a microcrystalline, colorless substance, which decomposed at 248 °C. Yield: 92%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.23 (s, 6H, H_3COCH_2), 3.40 (A_2B_2, 8H, $MeOCH_2CH_2$), 12.70 (br s, 4H, CO_2H), 13.15 (br s, 2H, NH). MS (EI, 70 eV): (*m/e*, rel intensity) 292 ($M - 3CO_2^+$, 10), 248 ($M - 4CO_2^+$, 36), 203 ($M - 4CO_2 - MeOCH_2^+$, 26), 171 ($M - 4CO_2 - MeOCH_2 - MeOH^+$, 100). UV/vis (DMSO): λ_{max} 258 (15 000), 277 (15 000), 343 (13 000). Anal. ($C_{18}H_{20}N_2O_{10}$) C,H,N.

4,4′-Bis(methoxyethyl)-2,2′-bipyrrole (10). Bipyrroletetracarboxylic acid **9** (8.5 g, 20 mmol) was decarboxylated in a sublimator at 230 °C (bath temperature)/0.1–0.2 Torr; 4.7 g (18.8 mmol) of **10** separated as a colorless, amorphous, or pale green crystalline solid. Yield: 94%. Bipyrrole **10** decomposed slowly above 75 °C and melted when rapidly heated at 98 °C. **10** was sensitive to acid and oxygen and was stored under argon at low temperature. ^{1}H NMR (acetone-d_6, 300 MHz): δ 2.64 (t, 4H, $MeOCH_2CH_2$), 3.27 (s, 6H, H_3COCH_2), 3.46 (t, 4H, $MeOCH_2CH_2$), 6.11 (m, 2H, H at C-3), 6.52 (m, 2H, H at C-5), 9.79 (br s, 2H, NH). MS (EI, 70 eV): (*m/e*, rel intensity) 248 (M^+, 82), 203 ($M - MeOCH_2^+$, 28), 171 ($M - MeOCH_2 - MeOH^+$, 100). UV/vis (CH_3CN): λ_{max} 343 (800), 284 (18 000).

5,5′-Diformyl-4,4′-bis(methoxyethyl)-2,2′-bipyrrole (5). Within 30 min, 6.14 g (40 mmol) of distilled phosphoryl chloride was added dropwise to a solution of 2.48 g (10 mmol) of **10** in 50 mL of absolute DMF under argon at 0 °C. The solution was heated to 60 °C for 1 h and then poured into a solution of 60 g of NaOAc in 480 mL of water. The mixture was stirred for 1 h at 85 °C, wherein the dialdehyde **5** precipitated out as yellow flakes. The suspension was cooled to 0 °C and filtered. The residue was washed with cold water. After drying over P_4O_{10} under vacuum, 2.58 g (8.5 mmol) of **5** resulted, which was recrystallized from THF. **5** decomposed at 186 °C without melting. Yield: 85%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.03 (t, 4H, $MeOCH_2CH_2$), 3.37 (s, 6H, H_3COCH_2), 3.62 (t, 4H, $MeOCH_2CH_2$), 6.54 (d, 2H, H at C-3), 9.7 (s, 2H, CHO), 12.27 (br s, 2H, NH). MS (EI, 75 eV): (*m/e*, rel intensity) 304 (M^+, 100), 272 ($M - MeOH^+$, 9), 227 ($M - MeOCH_2 - MeOH^+$, 19), 199 ($M - MeOCH_2 - MeOH - CO^+$, 19). UV/vis ($CH_2Cl_2$): λ_{max} 235 (9600), 271 (15 000), 381 (36 000). Anal. ($C_{16}H_{20}N_2O_4$) C,H,N.

2,7,12,17-Tetrakis(methoxyethyl)porphycene (1). Under argon, 16.5 mL (0.15 mol) of $TiCl_4$ was added over 10 min to a suspension of 19.6 g (0.3 mol) of zinc powder and 1.95 g (9.5 mmol) of CuCl in 800 mL of dry THF. The mixture was heated to reflux for 3 h. A solution of 1.83 g (6 mmol) of dialdehyde **5** in 600 mL of THF was added over 20 min while stirring vigorously. The reaction could be followed by TLC (silica/CH_2Cl_2). The suspension was stirred for 10 min at reflux and then cooled to 0 °C; 300 mL of 6% ammonia was added dropwise over 1 h. The reaction mixture was stirred with 600 mL of CH_2Cl_2 for 15 min and then filtered over Celite. The residue was extracted with another 200 mL of CH_2Cl_2, and the combined organic phases were washed three times with 300 mL of water. Following drying over $MgSO_4$, the solvent was rotoevaporated and the residue was chromatographed on Al_2O_3 (Brockmann, activity II–III, CH_2Cl_2). The first blue, red fluorescing fraction was collected and rechromatographed with CH_2Cl_2/ethyl acetate/methanol (100:20:1) on silica gel to separate off any potentially degraded porphycenes as a small blue fraction, eluted prior to 1. After evaporation of the solvent and crystallization from CH_2Cl_2/MeOH, 406 mg (0.75 mmol) of 1 was obtained as long, violet, metallically glistening needles. Mp 172 °C. Yield: 25%. ^{1}H

NMR ($CDCl_3$, 300 MHz): δ 3.11 (br s, 2H, NH), 3.60 (s, 12H, H_3COCH_2), 4.31 (s, 16H, $MeOCH_2CH_2$), 9.34 (s, 4H, H at C-3,6,13,16), 9.71 (s, 4H, H at C-9,10,19,20). MS (EI, 75 eV): (*m/e*, rel intensity) 542 (M^+, 81), 497 ($M - MeOCH_2^+$, 100), 452 ($M - 2MeOCH_2^+$, 20), 407 ($M - 3MeOCH_2^+$, 24), 375 ($M - 3MeOCH_2 - MeOH^+$, 3). UV/vis (CH_2Cl_2): λ_{max} 370 (140 000), 382 (sh, 95 000), 563 (35 000), 602 (33 000), 634 (47 000). Anal. ($C_{32}H_{38}N_4O_4$) C,H,N.

2,7-Bis(methoxyethyl)-12,17-dipropylporphycene (12). To a stirred suspension of 17.4 g (260 mmol) of zinc powder and 1.7 g (16.8 mmol) of anhydrous CuCl in 700 mL of dry, argon-saturated THF was added 14.2 mL (130 mmol) of $TiCl_4$ over 30 min. The mixture was heated to reflux for 3 h. A solution of 1.36 g (5 mmol) of diformyldipropylbipyrrole **11** and 1.52 g (5 mmol) of diformylbis(methoxyethyl)bipyrrole **5** in 300 mL of THF was added over 45 min. The mixture was stirred for 2 min at reflux and then cooled to 0 °C. At this temperature, 250 mL of 10% NH_3 was added over 1 h. The mixture was treated with 500 mL of THF, stirred under oxygen for 90 min, and filtered. The residue was extracted with 200 mL of CH_2Cl_2, and the combined organic layers were evaporated. The residue was taken up in CH_2Cl_2 and filtered over Celite. The residue was ground up and extracted with further CH_2Cl_2. The combined organic phases were evaporated, and the resulting dark blue residue was taken up in CH_2Cl_2 and chromatographed on alumina (Brockmann, activity II–III). All blue fractions were collected and rechromatographed with CH_2Cl_2/ethyl acetate (100:4) on silica gel. The first eluted compound consisted of 134 mg (0.28 mmol) of tetrapropylporphycene **13** followed by 280 mg (0.55 mmol) of **12** and 146 mg (0.27 mmol) of **1**, which eluted with pure ethyl acetate. **13** was recrystallized from CH_2Cl_2/hexane and **12** and **1** from CH_2Cl_2/MeOH, giving violet, metallically glistening needles. Mp (**12**) 142 °C. Yield: **12**, 11%; **1**, 5.5%; **13**, 5.5%. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.34 (t, 6H, $CH_2CH_2CH_3$), 2.41 (m, 4H, $CH_2CH_2CH_3$), 3.14 (br s, 2H, NH), 3.59 (s, 6H, CH_2OCH_3), 4.00 (t, 4H, $CH_2CH_2CH_3$), 4.31 (s, 8H, CH_2CH_2OMe), 9.27 (s, 2H, H-13,16), 9.34 (s, 2H, H-3,6), 9.71 (s, 4H, H-9,10,19,20). MS (EI, 70 eV): (*m/e*, rel intensity) 510 (M^+, 100), 481 ($M - C_2H_5^+$, 13), 466 ($M - C_3H_8^+$, 31), 466 ($M - MeOCH_2^+$, 88), 420 ($M - 2MeOCH_2^+$, 32), 255 (M^{2+}, 4). UV/vis (CH_2Cl_2): λ_{max} 370 (142 000), 382 (sh, 100 000), 562 (36 000), 601 (34 000), 633 (49 000). Anal. ($C_{32}H_{38}N_4O_2$) C,H,N.

2,7,12,17-Tetrakis(methoxyethyl)porphycenatonickel(II) (16). A suspension of 380 mg (0.7 mmol) of **1** in 120 mL of acetic acid was heated to reflux with 1.7 g (7 mmol) of $Ni(OAc)_2 \cdot 4H_2O$ for 5 h. On TLC (CH_2Cl_2/ethyl acetate, 4:1, silica gel), the nickel complex appeared as a less mobile, blue fraction without fluorescence. The mixture was poured into 600 mL of water and extracted three times with 150 mL of $CHCl_3$. The organic phases were washed twice with water, saturated aqueous $NaHCO_3$, and again twice with water and then dried over $MgSO_4$ and evaporated. After recrystallization from CH_2Cl_2/MeOH, 398 mg (0.67 mmol) of bluish-violet needles of **16** was obtained. Mp 182 °C. Yield: 95%. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.58 (s, 12 H, H_3COCH_2), 4.20 (A_2B_2, 16H, $MeOCH_2CH_2$), 8.82 (s, 4H, H at C-3,6,13,16), 9.28 (s, 4H, H at C-9,10,19,20). MS (DEI, 75 eV): (*m/e*, rel intensity) 599 (M^+, 15), 554 ($M - MeOCH_2^+$, 8). UV/vis (CH_2Cl_2): λ_{max} 265 (31 000), 387 (127 000), 603 (63 000). Anal. ($C_{32}H_{36}N_4O_4Ni$) C,H,N.

2-(Bromoethyl)-7,12,17-tris(methoxyethyl)porphycenatonickel(II) (17). At −78 °C, a solution of 419 mg (0.7 mmol) of **16** in 250 mL of dry CH_2Cl_2 under argon was treated with 62 mg (1 mmol) of boric acid and 126 mg (48 μL, 0.5 mmol) of BBr_3. The mixture was left to thaw over 8 h, and 50 mL of 5% $NaHCO_3$ was added dropwise over 10 min at 0 °C. The organic phase was separated and washed with saturated $NaHCO_3$ and water. After drying over $MgSO_4$, the solvent was evaporated and the residue purified by chromatography on silica gel (CH_2Cl_2/ethyl acetate/methanol, 80:20:1). The first three fractions contained various ether-split nickel porphycenes; the desired product (**17**) was eluted as the fourth fraction. Removal of the solvent and recrystallization from CH_2Cl_2/methanol afforded 159 mg (0.245 mmol) of blue-violet crystals of **17**, mp 186–189 °C. For the subsequent reaction of **17** → **15**, crystallization was not necessary. The fifth blue band yielded 209 mg (0.35 mmol, 50%) of the educt (**16**). Yield of **17**: 35%; yield based on recovered starting material, 70%. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.58, 3.59 (2 s, 9H, H_3COCH_2), 4.06, 4.10 (2 m, 16 H, $MeOCH_2CH_2$ and $BrCH_2CH_2$), 8.34, 8.45 (2 s, 2H, H at C-3,6), 8.55, 8.57 (2 s, 2H, H at C-13,16), 8.70 (A_2B_2, 2H, H at C-19,20), 8.95 (s, 2H, H at C-9,10). MS (EI, 75 eV): (*m/e*, rel intensity) 648 (M^+, 45), 603 ($M - MeOCH_2^+$, 54), 432 ($M - 3MeOCH_2 - Br^+$, 18), 418 ($M - 3MeOCH_2 - CH_2Br^+$, 21), 94 (CH_3Br^+, 35), 80 (HBr^+, 100). UV/vis (CH_2Cl_2): λ_{max} 264 (32 000), 387 (128 000), 605 (65 000).

2-(Bromoethyl)-7,12,17-tris(methoxyethyl)porphycene (15). **17** (97.2 mg, 0.15 mmol) was suspended in 12 mL of concentrated sulfuric acid and stirred for 10 min until a clear solution was produced. The solution was poured into 1 L of water and extracted three times with 150 mL of $CHCl_3$. The organic phases were washed three times with water and once with 1% $NaHCO_3$ and dried over $MgSO_4$. Following evaporation of the solvent and recrystallization from CH_2Cl_2/MeOH, 80 mg (0.135 mmol) of violet, glistening needles of **15** was obtained. Mp 164 °C. Yield: 90%. ^1H NMR (CF_3CO_2D, 300 MHz): δ 3.83, 3.84 (2 s, 9H, H_3COCH_2), 4.27 (t, 2 H, $BrCH_2CH_2$), 4.56, 4.66 (2 m, 14H, $MeOCH_2CH_2$ and $BrCH_2CH_2$), 10.03 (m, 4H, H at C-3,6,13,16), 10.54 (m, 4H, H at C-9,10,19,20). MS (EI, 75 eV): (*m/e*, rel intensity) 592 (M^+, 8), 547 ($M - MeOCH_2^+$, 2), 511 ($M - Br^+$, 1). UV/vis (CH_2Cl_2): λ_{max} 243 (13 000), 370 (133 000), 382 (95 000), 564 (34 000), 603 (32 000), 635 (45 000). Anal. ($C_{31}H_{35}N_4O_3Br$) C,H,N,Br.

2-Vinyl-7,12,17-tris(methoxyethyl)porphycene (18). All reagents and solvents were saturated with argon, and all steps were carried out under subdued light; 71 mg (0.12 mmol) of **15** was dissolved in 50 mL of THF and treated with 1.8 g (1.8 mL, 12 mmol) of DBU. The reaction solution was stirred at 40 °C for 90 min in the dark and treated with 150 mL of CH_2Cl_2. The mixture was poured into 100 mL of 5% HCl, shaken with 2% $NaHCO_3$, and washed with water. The organic phase was evaporated in a slight vacuum, and the residue was chromatographed on Al_2O_3 (Brockmann, activity II-III, CH_2Cl_2). Following crystallization, 52.6 mg (0.103 mmol) of **18** was obtained from the single mobile blue fraction as needle-shaped crystals. Mp 115–120 °C dec. Yield: 86%. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.05 (br s, 2H, NH), 3.40, 3.59, 3.95 (3 s, 9H, H_3COCH_2), 4.28 (m, 12H, $MeOCH_2CH_2$), 5.95 (dd, 1H, $CH=CH_2$ cis), 6.60 (dd, 1H, $CH=CH_2$ trans), 8.25 (dd, 1H, $CH=CH_2$), 9.25, 9.26 (2 s, 3H, H at C-6,13,16), 9.53 (s, 1H, H at C-3), 9.60 (AB, 2H, H at C-9,10), 9.69 (AB, 2H, H at C-19,20). MS (FAB, 75 eV): (*m/e*, rel intensity) 510 (M^+, 100), 465 ($M - MeOCH_2^+$, 23). UV/vis (CH_2Cl_2): λ_{max} 373 (98 000), 572 (26 000), 613 (28 000), 643 (33 000). HR-MS ($C_{31}H_{34}N_4O_3$): calcd, 510.262; found, 510.262.

2^3,2^4-Bis(methoxycarbonyl)benzo[2,3]-7,12,17-tris(methoxyethyl)porphycene (19). Vinylporphycene **18** (51 mg, 0.1 mmol) was dissolved in 7 mL of dimethyl acetylenedicarboxylate (DMAD) and heated to 75 °C under stirring in an argon atmosphere for 90 min. Excess DMAD was removed at 50 °C/0.2 torr, and the residue was chromatographed on silica gel (CH_2Cl_2/glacial acetic acid, 50:1–20:1). The second (main) band was collected and washed with saturated $NaHCO_3$ and water, dried over $MgSO_4$, and evaporated. The residue was washed twice with cold ether/pentane (3:1) and recrystallized twice from CH_2Cl_2/MeOH to give 39 mg (60 μmol) of **19** as a violet microcrystalline solid. Mp 209–210 °C. Yield: 60%. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.55 (br s, 1H, NH), 1.87 (br s, 1H, NH), 3.55 (s, 3H, H_3COCH_2 at C-17), 3.61 (2s, 6H, H_3COCH_2 at C-7,17), 4.11–4.32 (3m, 12H, $MeOCH_2CH_2$), 4.16 (s, 3H, CO_2CH_3 at C-2^3), 4.41 (s, 3H, CO_2CH_3 at C-2^4), 8.55 (d, 1H, H at C-2^2, ^3J = 8.4 Hz), 9.09 (s, 1H, H at C-13), 9.14 (d, 1H, H at C-16), 9.17 (d, 1H, H at C-2^1), 9.29 (AB, 1H, H at C-19), 9.55 (d, 1H, H at C-6), 9.60, 9.67 (2AB, 2H, H at C-9,-10), 9.72 (AB, 1H, H at C-20) MS (EI, 75 eV): (*m/e*, rel intensity) 650 (M^+, 86), 605 ($M - MeOCH_2^+$, 100), 560 ($M - 2MeOCH_2^+$, 15), 515 ($M - 3MeOCH_2^+$, 8. UV/vis (CH_2Cl_2): λ_{max} 255 (24 000), 396 (101 000), 576 (32 000), 612 (60 000), 639 (42 000). HR-MS ($C_{37}H_{38}N_4O_7$): calcd, 650.273; found, 650.271 (±0.005).

2^3-Carboxy-2^4-(methoxycarbonyl)benzo[2,3]-7,12,17-tris(methoxyethyl)porphycene (20). **19** (23 mg, 35 μmol)

in 75 mL of THF was treated with a solution of 480 mg (20 mmol) of LiOH in 75 mL of water and stirred for 5 h at room temperature in the dark. The solution was brought to pH 2–3 with HCl, and the organic layer was separated and extracted twice with 200 mL of 1% $NaHCO_3$/MeOH (2:1). The combined aqueous phases were acidified and extracted twice with 50 mL of CH_2Cl_2/MeOH (2:1). The organic phases were evaporated, and the residue was chromatographed on silica gel (CH_2Cl_2/MeOH). The first band contained 2.1 mg (9%) of starting material (**19**), and the second band yielded, after evaporation and recrystallization from CH_2Cl_2/MeOH, 14.5 mg (23 μmol) of **20** as a blue microcrystalline powder. MP 194 °C. Yield: 65%. HPLC: purity, >96%; column, Nucleosil C_{18} (5 μm) (Machery-Nagel, Germany); system I, CH_3CN:0.1 M phosphate buffer, pH 7.8:0.1% tetrabutylammonium hydroxide (40:60:0.1), t_R 10.9 min; system II, MeOH, t_R 2.1 min. ^1H NMR ($CDCl_3$/CF_3CO_2D, 300 MHz): δ 3.44 (s, 3H, H_3COCH_2 at C-7), 3.68 (s, 3H, H_3COCH_2 at C-12), 3.83 (s, 3H, H_3COCH_2 at C-17), 4.23 (A_2B_2, 4H, $MeOCH_2CH_2$ at C-7), 4.46 (A_2B_2, 4H, $MeOCH_2CH_2$ at C-12), 4.53 (s, 3H, CO_2CH_3 at C-2[4]), 4.64 (A_2B_2, 4H, $MeOCH_2CH_2$ at C-17), 9.01 (AB, 1H, H at C-2[2], ^3J = 8.3 Hz), 9.55 (AB, 1H, H at C-2[1]), 9.37 (s, 1H, H at C-6), 9.71 (s, 1H, H at C-13), 9.80 (s, 1H, H at C-16), 10.01 (AB, 2H, H at C-9,10, ^3J = 11.4 Hz), 10.18 (AB, 1H, H at C-19, ^3J = 11.4 Hz), 10.49 (AB, 1H, H at C-20). ^{13}C NMR ($CDCl_3$/CF_3CO_2D, 75.5 MHz): δ 27.35 (C12α), 27.51 (C17α), 27.56 (C7α), 54.83 (CO_2CH_3 at C-2[4]), 57.98 (H_3COCH_2 at C-7), 58.35 (H_3COCH_2 at C-12), 58.74 (H_3COCH_2 at C-17), 72.95 ($MeOCH_2CH_2$ at C-7,17), 73.02 ($MeOCH_2CH_2$ at C-12), 111.63, 112.15 (C9,10), 115.32 (C19), 115.55 (C20), 120.23 (C6), 123.55 (C13), 124.32 (C2[1]), 124.52 (C16), 127.40 (C2[3]), 129.02 (C14), 131.62 (C2[4]), 131.68 (C15), 132.32 (C2[2]), 133.52 (C3), 133.70 (C8), 134.21 (C5), 137.84 (C12), 139.11 (C11), 139.59 (C7), 139.99 (C18), 143.22 (C4), 144.12 (C17), 147.62 (C2), 149.27 (C1), 171.60 (CO_2H at C-2[3]), 173.27 (CO_2Me at C-2[4]). MS (FAB): (*m/e*, rel intensity) 636 (M^+, 48), 591 ($M - MeOCH_2^+$, 16). UV/vis ($CHCl_3$/MeOH, 9:1): λ_{max} 396 (89 000), 576 (27 000), 610 (55 000), 636 (36 000).

2-(Hydroxyethyl)-7,12,17-tris(methoxyethyl)porphycene (14). The reaction was carried out under a dry argon atmosphere with carefully dried equipment and solvents; 70 μL (0.74 mmol) of dry boron tribromide in 70 mL of CH_2Cl_2 was added during 60 min to a stirred solution of 542 mg (1 mmol) of **1** in 150 mL of CH_2Cl_2 at −30 °C. Over 16 h, the mixture was allowed to reach room temperature and 25 mL of 8% $NaHCO_3$ was added. The precipitate was filtered over Celite and washed with $NaHCO_3$ solution and water. The remaining solid was extracted with methanol. The organic phase of the filtrate was washed with $NaHCO_3$ solution and twice with water and then combined with the methanol extract. The crude product was preadsorbed on 15 g of Al_2O_3 and fractionated by silica gel chromatography (dichloromethane/ethyl acetate/ethanol, 100:10:1). After a forerun of 300–360 mg (depending on remaining water in solvent and equipment) of educt **1** (50–65%), the (hydroxyethyl)tris(methoxyethyl)-porphycene **14** was eluted and crystallized from CH_2Cl_2/MeOH (1:1) to afford 93 mg (0.18 mmol) of dark violet cubes, mp 144–146 °C. The isomeric dialcohols (53 mg, 10–12%) and the trialcohol (20 mg, 4%) were eluted with chloroform/methanol (8:1). Yield of **14**: 18%. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.91 (br s, 1H, OH), 3.12 (br s, 2H, NH), 3.59 (3s, 9H, H_3COCH_2), 4.27 (t, 2H, $HOCH_2CH_2$), 4.31 (s, 12 H, $MeOCH_2CH_2$), 4.53 (m, 2H, $HOCH_2CH_2$), 9.33, 9.34, 9.35, 9.36 (4 s, 4H, H at C-3,6,-13,16), 9.70 (2AB, 4H, H at C-9,10,19,20). MS (EI, 75 eV): (*m/e*, rel intensity) 528 (M^+, 80), 497 ($M - HOCH_2^+$, 10), 483 ($M - CH_3OCH_2^+$, 100). UV/vis (CH_2Cl_2): λ_{max} 370 (134 000), 563 (33 600), 602 (31 200), 635 (43 900). Anal. ($C_{31}H_{38}N_4O_4$) C,H,N.

2,7-Bis(hydroxyethyl)-12,17-dipropylporphycene (21). To a solution of 255 mg (0.5 mmol) of porphycene diether **12** in 150 mL of dry CH_2Cl_2 under argon was added 189 μL (0.5 g, 2 mmol) of BBr_3 in 10 mL of CH_2Cl_2 at −78 °C. The green solution was left to reach 0 °C over 2 h and hydrolized with 30 mL of 5% $NaHCO_3$ over 10 min, 200 mL of THF was added, and the mixture was filtered. The organic layer was separated, washed with water, and evaporated. The residue was chromatographed on silica gel (CH_2Cl_2/THF, 4:1). The fifth (main) band yielded 168 mg (0.35 mmol) of violet needles **21** after crystallization from ethyl acetate. Mp 236 °C. Yield: 70%. ^1H NMR (DMSO-d_6, 300 MHz): δ 1.28 (t, 6H, $CH_2CH_2CH_3$), 2.34 (m, 4H, $CH_3CH_2CH_2$), 3.03 (br s, 2H, NH), 3.91 (t, 4H, $CH_2CH_2CH_3$), 4.20 (t, 4H, CH_2CH_2OH), 4.37 (m, 4H, CH_2CH_2OH), 5.16 (t, 2H, OH), 9.60 (s, 2H, H at C-13,16), 9.65 (s, 2H, H at C-3,6), 9.86 (s, 4H, H at C-9,10,19,20). MS (EI, 70 eV): (*m/e*, rel intensity) 482 (M^+, 100), 453 ($M - C_2H_5^+$, 45), 452 ($M - CH_2O^+$, 37), 451 ($M - HOCH_2^+$, 90), 420 ($M - 2HOCH_2^+$, 25), 181 ($M - 2HOCH_2 - 2C_2H_5^+$, 63). UV/vis (CH_2Cl_2): λ_{max} 369 (138 000), 381 (sh, 99 000), 562 (36 000), 601 (34 000), 633 (48 000). Anal. ($C_{30}H_{34}N_4O_2$) C,H,N.

Biological Studies. Photofrin was supplied by Lederle, Wolfrathshausen, FRG, and was diluted with MEM for incubation experiments. To calculate molar concentrations, the molecular weight of the porphyrin species in Photofrin was approximated with 598.7 (hematoporphyrin).

For cell culture experiments, Hank's balanced salt solution was made up from pro analysi salts and aqua bidest. Solution A was 142 mM NaCl, 5 mM KCl, 0.4 mM Na_2HPO_4, 0.4 mM KH_2PO_4, and 0.06 mM phenol red. Earl's minimum essential medium (MEM) including penicillin and streptomycin, trypsin/EDTA (0.25%/1% solution), and fetal calf serum (FCS) were purchased from Biochrom, Berlin, FRG. Trypan blue, 0.5% solution, was from Boehringer, Mannheim, FRG. Laser light irradiation was performed with a dye laser (Model 375B; Spectra-Physics, Mt. View, CA; dye: kiton red; Radiant Dyes, Wermelskirchen, FRG) pumped with the 514 nm line of an argon laser (Model 2035; Spectra-Physics, Mt. View, CA). Light intensity was measured using a Model 210 powermeter (Coherent, Palo Alto, CA). Fluorescence spectra of cell extracts were recorded on a Shimadzu RF 540 fluorometer equipped with an extended red sensitive S20 photocathode (Hammamatsu).

Liposomes. For biological experiments, porphycenes were incorporated in unilamellar liposomes. These were prepared from dipalmitoylphosphatidylcholine (DPPC) or dioleylphosphatidylcholine (DOPC), both ≥99% purity, from Sigma, Deisenhofen, Germany. DPPC liposomes were used for the phototoxicity study and the flow cytometric experiments to rule out oxidation of liposomal lipid molecules during irradiation; the saturated lipid is inert to oxidation. DOPC liposomes were employed in kinetic experiments with dye extraction, where oxidative stability was not an issue, for their long-time thermodynamic stability (discussed in ref 29 and literature cited therein). Thus, they allow prolonged incubation times (and possibly cooling experiments) without special precautions. The experimental details of the DOPC liposome preparation have been described earlier[29] and can be summarized briefly as follows: a film produced by coevaporation of phosphatidylcholine and porphycene solutions was taken up in PBS to yield a 0.1 M lipid suspension; samples were sonicated with a Branson probe sonicator and subjected to 0.2 μm sterile filtration after 24 h annealing time; all steps were carried out under argon protective gas and subdued light. The DOPC liposomes thus formed have a size between (100 ± 30) and (127 ± 77) nm (SD), and porphycenes are incorporated with ≥65% incorporation efficiency.[29] The preparation of DPPC liposomes[7,23] required the following modifications of the recipe given in ref 29: the ratio DPPC/porphycene was 100:1, and after sonication, liposomes were kept above 40 °C, filtered, and used immediately, to avoid precipitation. All liposome preparations were checked before use by UV/vis spectroscopy of diluted aliquots. Fluorescence maxima of porphycenes in liposomes were ≤8 nm from the longest wavelength UV/vis absorption maxima. For incubation with cells, liposome suspensions were diluted with MEM to sensitizer concentrations given in the text.

Cultivation of Cells and Incubation with Sensitizers. Experiments were carried out using SSK2 cells (a murine cell line derived from a methylcholandrene-induced fibrosarcoma of a C3H mouse[33]), kindly provided by Dr. J. Kummermehr, GSF, München, FRG. The cells were routinely maintained by serial culture in MEM supplemented with 5% FCS and 1% antibiotics at 37 °C in a 5% CO_2 atmosphere. The cells exponentially grew with a doubling time of 22 h. When

suspended by trypsination, cells had a spherical shape and a mean diameter of 16 μm as determined in a CASY 1 (Schärfe, Reutlingen, FRG) electronic cell counter. Solution A and trypsine treatment was performed at 37 °C on a heating plate.

For all experiments, 2.5×10^5 cells were plated out in plastic petri dishes (Nunclon, 3 cm diameter; Nunc, Roskilde, Denmark) using 1 mL of MEM/5% FCS and left to attach and grow for 18 h, resulting in 3×10^5 cells/dish. The incubation medium was removed, and 1 mL of sensitizer solutions in MEM was added and the mixture incubated for appropriate time intervals at 37 °C/5% CO_2. For each data point, at least five petri dishes were employed, and each experiment included five dishes receiving MEM only (control).

Phototoxicity Studies. Cells were incubated for 4 h, washed once with PBS, and covered with 1 mL of PBS for light treatment; 20 mW/cm^2 was applied to each dish for 250 s, resulting in 5 J/cm^2. After irradiation, the PBS was carefully aspirated, 1 mL of MEM/10% FCS was added, and cells were kept at 37 °C/5% CO_2 for 18 h. Cells were washed with 1 mL of solution A, which was kept on the cells for 3 min. Then, 0.5 mL of trypsine/EDTA was added (3 min), and the cells were suspended after addition of 1 mL of MEM/5% FCS. An aliquot of the suspension (1 mL) was mixed with 0.25 mL of trypan blue solution, and unstained cells were counted in Neubauer chambers (eight countings per dish). Cell viability was expressed as percent of control, and LD_{50} values were obtained from probit analysis.[24] Laser light treatment alone had no effect on cell viability in control groups. Experiments at selected sensitizer concentrations employing a colony-forming assay to determine cell survival yielded similar results to the trypan blue test used, though with much higher standard deviation (data not shown).

Flow Cytometry. Cells were incubated at 37 °C/5% CO_2 with 2 μM porphycene solutions (5 μM for **20**, due to its lower absorption at the excitation wavelength). They were then washed with solution A and suspended by treatment with trypsine/EDTA and MEM/FCS as described in the preceding section. For each flow cytometric measurement, 100 μL of the cell suspension was mixed with 10 μL of a suspension of fluorescence beads (0.2 μm diameter, Fluoresbrite yellow-green; Polyscience, WA) and filtered (35 μm pore size). Dual laser flow cytometry of 10^4 cells was performed using a FACStar$^+$ flow cytometer (Becton Dickenson, San Jose, CA). Data recording was done with a HP 9000 Series 300 computer in list mode using the FACStar$^+$ research software. The first laser (Innova 90; Coherent, Palo Alto, CA) was adjusted to the 488 nm line and the second laser (Innova 100) to the UV multilines (351.1–363.8) nm. Pulse height of fluorescence intensity and forward scatter were detected simultaneously. Fluorescence of beads was detected employing a band-pass filter, 530BP10 (Becton Dickenson). Porphycene fluorescence was detected using an OG 590 nm filter in combination with a KV 550 nm filter (Schott, Germany). Cellular porphycene fluorescence was expressed as the ratio to the fluorescence of reference beads, to obtain values independent of instrumental fluctuations. Discrimination between beads and particles was achieved by setting gates around the beads and the cells in the dot plot of pulse height of fluorescence intensity versus pulse height of forward light scatter intensity.

Porphycene Extraction from Cells. Cells were incubated with 2 μM porphycene solutions, washed, and suspended after incubation as described in the preceding section. Cell density was determined from aliquots of each sample, and 1 mL of the remaining suspension was centrifuged at 1000*g* for 5 min. The supernatant was decanted from the cell pellet, from which porphycenes were extracted by a modification of a procedure that had been established for the extraction of serum, erythrocytes, and animal tissue.[10] In control experiments, this procedure gave recovery yields ≥(95 ± 5)% for every porphycene employed. The extraction procedure was optimized to give maximum recovery with as little background absorption and fluorescence as possible from the cells and to ensure compatibility with an internal standard. The optimization study[10] had involved aqueous detergent solutions, solid phase extraction media, and organic solvents like dichloromethane, chloroform, THF, 2-propanol, methanol, acetonitrile, and DMSO. Binary and ternary mixtures and subsequent treatment with these media were studied. In the chosen procedure, cell pellets were treated with 0.2 mL of methanol followed by short agitation and addition of 1.8 mL of acetonitrile containing 95 nM diphenylanthracene (Sigma, Deisenhofen, FRG) as internal fluorescence standard. After sonication (Branson sonicator W 250 operating at 40 W for 10 s) and centrifugation at 2500*g* for 10 min, porphycene fluorescence was measured in supernatants ($\lambda_{exc} = 370$ nm, $\lambda^{max}_{det} = 640 \pm 10$ nm) and concentrations were read against diphenylanthracene ($\lambda_{det} = 640 \pm 10$ nm) and concentrations were read against diphenylanthracene ($\lambda_{det} = 429$ nm) according to calibration plots. Kinetics of cellular concentrations (mean ± SD) were subjected fit procedures testing mono- and multiexponential and power functions, using the Marquardt algorithm.

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Supplementary Material Available: Analytical data (combustion analyses and ^{13}C NMR and IR data) for **1**, **2a,b**, **4–10**, **12**, and **14–21** (4 pages). Ordering information is given on any current masthead page.

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