Synthesis and Biolocalization of Water-Soluble Sapphyrins

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The synthesis, characterization, and biolocalization properties of new, fully water-soluble sapphyrins is reported. These systems, which display light absorbing features that make them of potential interest as photodynamic therapy (PDT) sensitizers, were found to localize selectively in pancreatic carcinoma tissue in a xenographic murine model. In this model, the concentration ratios for malignant tissue to surrounding muscle tissue were found to be as high as 280 in the most favorable case. The concentrations of the water-soluble sapphyrins reported here, as well as a porphyrin control system, were determined as a function of time and tissue type.

Introduction

Photodynamic therapy (PDT) has attracted increasing interest as a novel approach for cancer treatment.[1−3] One of the attractive features of PDT is that it offers the potential for greater selectivity for inactivating tumor cells preferentially over healthy cells, as only those cells that are simultaneously exposed to both the photosensitizer and light experience a cytotoxic effect. This selectivity is achieved by a combination of preferential uptake of the photosensitizer into diseased tissue and restricting light exposure to such sites.

Photofrin, a mixture of various multimeric hematoporphyrin derivatives, is currently the only photosensitizer widely approved for the treatment of various types of cancers.[1−5] While Photofrin has proven effective, there are features that could be improved upon by new photosensitizers. First, it is desirable from a drug testing and approval standpoint to produce single isolable compounds, rather than multicomponent mixtures. The development of PDT agents that absorb light above 630 nm (where bodily tissues are more transparent) is also desirable because it would allow a greater depth of penetration and hence potentially greater per photon destruction of malignant tissues. Another goal is the development of PDT agents that display increased selectivities for malignant tissue relative to other tissues. Finally, compounds with low long-term retention in normal tissues, especially the skin, would be desirable since that might obviate the prolonged cutaneous phototoxicity that plagues PDT patients treated with Photofrin.[1,2] Toward these ends, a variety of photosensitizers, related to chlorins, bacteriochlorins, porphycenes, phthalocyanines, and expanded porphyrins have been developed and evaluated as putative PDT agents in recent years.[2−4,6−10] We have largely focused our own efforts on expanded porphyrin-type systems.[3,7,11−16]

Expanded porphyrins, polypyrrolic macrocycles that contain more coordinating heteroatoms, larger central binding cores, and/or more extensive π-electron conjugation pathways than the porphyrins, are attractive as potential PDT agents because they can combine many of the advantageous features of porphyrins (e.g., tumor biolocalization, chemical stability, singlet oxygen generation, etc.) with important properties that the porphyrins do not possess (e.g., red-shifted absorption maxima).[3,6,7] Unlike porphyrins, however, expanded porphyrins are not naturally occurring. In fact, the chemistry of these compounds dates to 1966 when R. B. Woodward reported the serendipitous discovery of sapphyrin,[17,18] a pentapyrrolic expanded porphyrin containing one bipyrrolic linkage (cf. the core component of structures **1**−**5**; Figure 1). Since then, the sapphyrins have remained one of the most widely studied of expanded porphyrin systems.[13−16,19−25] In the course of studying the phosphate binding properties of sapphyrins in protic media, we became aware of several features that led us to consider that they might be effective PDT agents.[19−21] Sapphyrins absorb light in the red portion of the visible spectrum, displaying a lowest energy Q-band like absorption maximum at $\lambda_{max} = 675$ nm in aqueous media and at even longer wavelengths in less polar environments. They also generate singlet oxygen in a good quantum yield[13] and have been found to catalyze the photocleavage of DNA effectively.[14−16] Meanwhile, Chandrashekar and co-workers have examined the uptake of a sapphyrin derivative into human erythrocytes tissue and found that its rate of release from such tissues is faster than what is seen for Photofrin.[22]

These encouraging results led us to examine a series of sapphyrins (compounds **1**−**5**) to study the effect of

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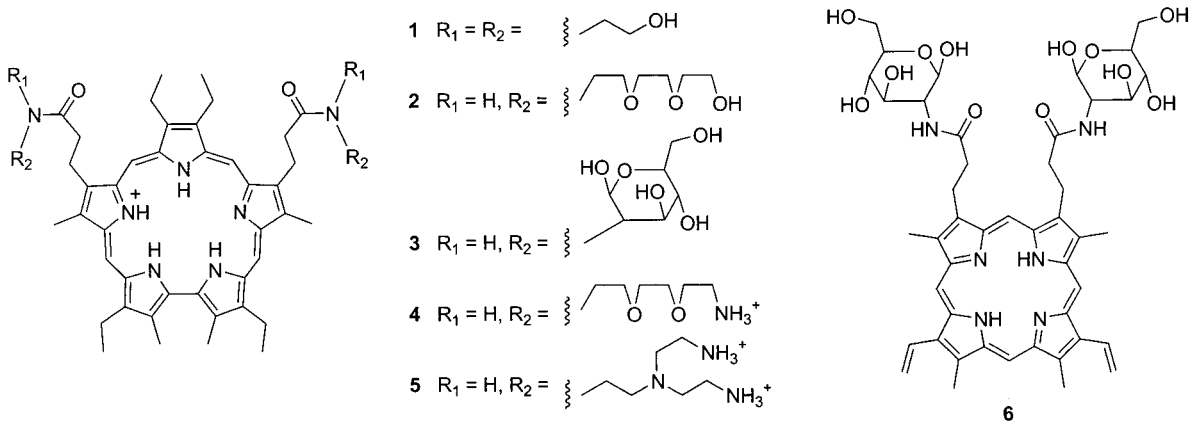
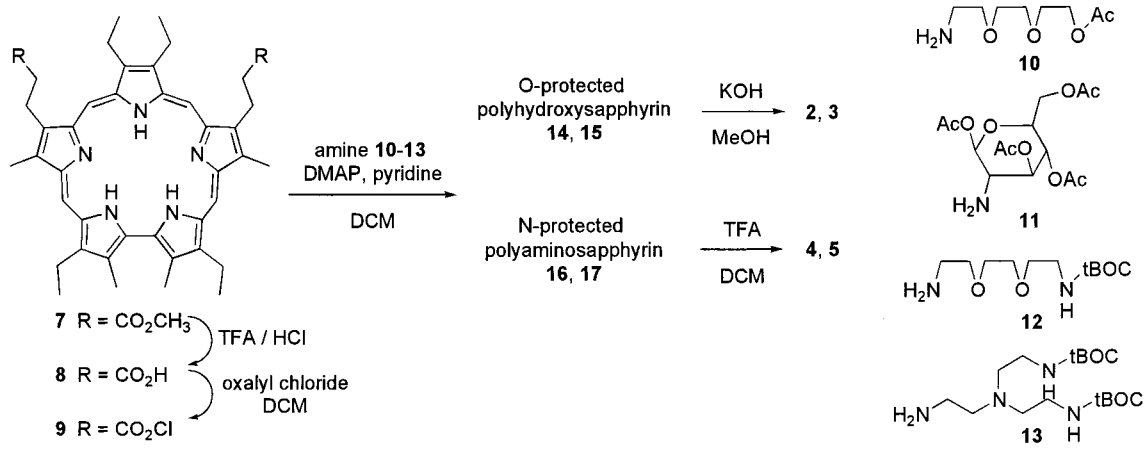


Figure 1. Structures of the water-soluble sapphyrins used in this study (**1**−**5**) and a corresponding porphyrin control **6**.

Scheme 1



water-solubilizing appendages and overall charge on the in vivo uptake and release in various quintessential mammalian tissues, including xenographic human pancreatic adenocarcinomas. In a recent study using water soluble sapphyrin polyamines, including **4** and **5**, concentrations were found to be as much as 3 orders of magnitude higher in malignant tissue than in liver, kidney, and muscle tissue.[23] To build on this work, we wanted to examine more fully the effect of different water-solubilizing groups as well as overall macrocycle charge on the biodistribution of sapphyrins. In this paper, we report the synthesis of new sapphyrin derivatives **2** and **3**, as well as a full description of the synthesis of **4** and **5**, which has not yet been published (cf. Figure 1). We also report the biolocalization properties of these sapphyrins, as well as the known water-soluble sapphyrin **1** and the control porphyrin **6**, in nu/nu nude mice containing implanted xenographic pancreatic cancer tissue monitored over a period of 1−9 days by tissue extraction and UV−Vis absorption spectroscopy.

Chemistry

Sapphyrin **1**[21] and porphyrin **6**[26] were prepared according to literature procedures. The general procedure for the preparation of water-soluble sapphyrins **2**−**5** is outlined in Scheme 1. The sapphyrin dimethyl ester **7**, which was also prepared according to the literature,[21] was hydrolyzed to the diacid **8** by heating at 60 °C in a 1:1 mixture of trifluoroacetic acid and hydrochloric acids for 12 h. Conversion of the sapphyrin diacid **8** to the activated acid chloride **9** was accomplished by stirring with oxalyl chloride in dry dichloromethane for 2 h at room temperature.[24,25] The acid chloride was then reacted with the O-protected aminopoly(ethylene glycol) **10**, O-protected glucopyranose **11**, and the partially N-protected polyamines **12** and **13** in dichloromethane, as depicted in Scheme 1. These coupling reactions were catalyzed by the addition of DMAP and pyridine. Following purification by column chromatography, the acetate and *t*-BOC protecting groups were removed via reaction with KOH in methanol and TFA in dichloromethane, respectively.

Biological Results and Discussion

The specific sapphyrins chosen for this study (cf. Figure 1) were designed to allow the effect of different polar substituents on the biophysical properties of sapphyrin to be evaluated efficiently. At physiological pH, the sapphyrin core exists predominantly in the form of the monoprotonated monocation.[19,21] Thus, at neutral pH, compounds **1**−**3** have an overall charge of +1. By appending primary amines onto the sapphyrin backbone, it proved possible to introduce additional cationic charge into systems **4** and **5**, giving them an estimated overall charge at neutral pH of +3 and +7, respectively.

The distributions of compounds **1**−**6** in mouse tissues and blood at 1, 3, 6, and 9 days following injection are summarized in Table 1 and shown graphically in Figure 2. While a wide range of biodistributions was observed,

Table 1. Concentrations of Sapphyrins **1**−**5** and Porphyrin **6** in Mouse Tissues (μg g^{-1})

		concentration by tissue type								
drug	days	tumor	liver	lung	kidney	blood	heart	spleen	brain	muscle
1	1	109 ± 16	1.42 ± 0.19	0.48 ± 0.09	0.44 ± 0.07	0.50 ± 0.17	0.95 ± 0.06	1.29 ± 0.07	0.24 ± 0.05	1.02 ± 0.06
	3	220 ± 40	1.50 ± 0.12	0.71 ± 0.16	0.33 ± 0.06	0.90 ± 0.04	0.96 ± 0.02	1.39 ± 0.10	0.27 ± 0.04	0.76 ± 0.16
	6	130 ± 30	1.2 ± 0.3	0.71 ± 0.04	0.46 ± 0.02	0.50 ± 0.12	0.76 ± 0.05	0.96 ± 0.06	0.29 ± 0.07	0.71 ± 0.08
2	1	22 ± 5	14 ± 4	13 ± 2	3.1 ± 1.3	7.0 ± 1.4	8 ± 2	4.6 ± 1.4	2.7 ± 0.4	10 ± 2
	3	130 ± 20	61 ± 13	11 ± 3	59 ± 10	9.3 ± 1.2	7.8 ± 1.6	11 ± 3	4.3 ± 0.3	9.3 ± 1.6
	6	180 ± 30	16 ± 3.2	19 ± 5.3	52 ± 7	3.4 ± 1.2	4.8 ± 1.2	14 ± 2	4.3 ± 1.3	3.4 ± 0.7
3	1	32 ± 6	0.29 ± 0.05	4.9 ± 1.2	1.1 ± 0.2	1.44 ± 0.17	5.4 ± 0.71	3.6 ± 0.8	0.55 ± 0.08	8.0 ± 1.5
	3	66 ± 10	0.43 ± 0.03	8 ± 2	1.9 ± 0.3	2.0 ± 0.6	9 ± 2	7.8 ± 1.0	0.77 ± 0.19	21 ± 5
	6	330 ± 30	0.37 ± 0.04	6 ± 2	1.5 ± 0.3	2.2 ± 0.5	6.4 ± 1.9	8.0 ± 0.3	0.75 ± 0.06	17 ± 3
	9	23 ± 3	0.47 ± 0.03	8 ± 2	2.8 ± 0.6	1.5 ± 0.5	6 ± 5	9.7 ± 0.4	0.52 ± 0.07	11 ± 3
4	1	4.2 ± 1.4	0.21 ± 0.08	1.7 ± 0.7	0.64 ± 0.17	1.69 ± 0.16	2.0 ± 0.4	1.3 ± 0.5	0.50 ± 0.14	1.9 ± 0.9
	3	114 ± 13	25 ± 5	19 ± 3	6.8 ± 1.6	3.02 ± 0.15	15.5 ± 1.6	11 ± 3	5.0 ± 0.7	29 ± 3
	6	51 ± 10	4.2 ± 1.2	7 ± 3	3.8 ± 0.5	2.45 ± 0.19	9.2 ± 0.7	3.3 ± 0.8	2.4 ± 0.5	45 ± 11
5	1	58 ± 14	1.03 ± 0.09	2.5 ± 1.8	2.8 ± 1.0	0.95 ± 0.08	3.1 ± 0.6	4.1 ± 1.6	0.87 ± 0.12	3.6 ± 1.2
	3	89 ± 11	13 ± 4	13 ± 4	5.7 ± 1.8	3.4 ± 1.0	10.7 ± 0.5	8 ± 4	0.40 ± 0.19	36 ± 6
	6	49 ± 9	10 ± 3	3.2 ± 1.2	1.7 ± 0.5	1.17 ± 0.17	5.2 ± 0.8	3.9 ± 1.5	0.16 ± 0.02	22 ± 3
6	1	34 ± 9	1.10 ± 0.14	2.0 ± 0.3	0.7 ± 0.2	1.09 ± 0.12	2.7 ± 0.7	22 ± 9	0.42 ± 0.12	3.5 ± 0.4
	3	131 ± 3	0.80 ± 0.130	1.7 ± 0.6	3.1 ± 0.3	0.90 ± 0.12	1.45 ± 0.12	16 ± 4	0.41 ± 0.06	4.2 ± 0.5
	6	260 ± 20	1.32 ± 0.10	1.4 ± 0.2	4.3 ± 0.7	0.98 ± 0.12	1.7 ± 0.4	12 ± 3	0.32 ± 0.08	5.1 ± 1.7
	9	37 ± 7	0.96 ± 0.15	2.3 ± 1.1	0.51 ± 0.17	0.8 ± 0.3	2.4 ± 0.3	9 ± 3	0.37 ± 0.15	3.6 ± 1.1

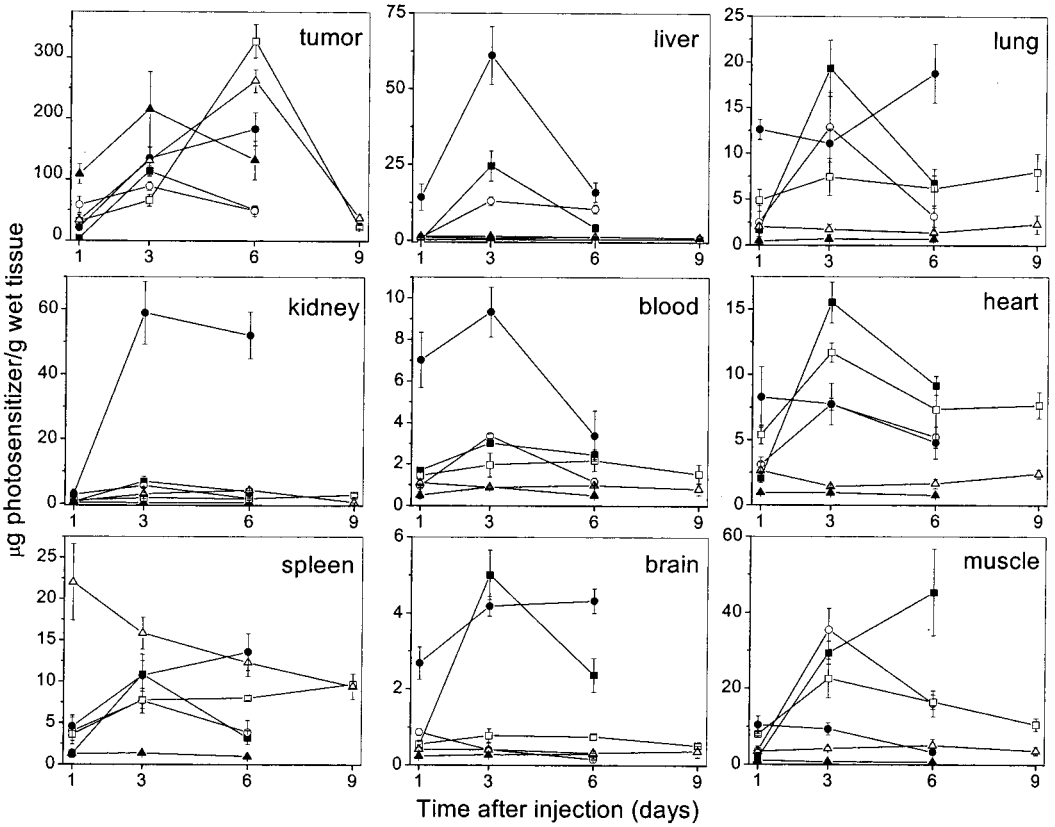


Figure 2. Concentrations of potential photosensitizers **1** (−▲−), **2** (−●−), **3** (−□−), **4** (−■−), **5** (−○−), and **6** (−△−) in different tissues of nude mice at different times following 3 days of injections (15 μmol kg^{-1} day^{-1}). Five animals were used for each time point and standard deviations are indicated with error bars. The tumor used was a xenographic pancreatic carcinoma; see text for details.

it was found that all six compounds displayed a higher degree of localization in the xenographic tumor tissue than in normal tissues and blood. Further, among the normal tissues, the concentrations in all cases was lowest in the brain, a finding that presumably reflects the exclusion of sapphyrins and porphyrin by the blood−brain barrier.

As implied above, all of the sapphyrins were found to concentrate effectively in the tumor (in the range of 90−330 μg g^{-1}) following a standard administration of 15 μmol kg^{-1} per day for 3 days. Sapphyrin **3** exhibited maximal tumor content (327 μg g^{-1}), whereas sapphyrin **5** showed minimal content in tumor tissue (89 μg g^{-1}), both following standard administration. The greatest sapphyrin content was observed at day 3 after the last injection for compounds **1** and **4**. By contrast, maximal tumor content was seen at day 6 for the other compounds. The ratio of photosensitizer content in the tumor relative to the concentration in surrounding normal tissue (i.e., muscle), referred to as T/M, was found to be roughly 280 to 1 for sapphyrin **1** and on the order of 20−54 to 1 for the other monocationic sapphy-

Table 2. Tumor to Muscle (T/M) and Tumor to Liver (T/L) Ratios of Potential Photosensitizers **1**−**6** at the Time of Maximal Tumor Concentration

drug	days	T/M	T/L
1	3	280 ± 80	140 ± 30
2	6	54 ± 14	11 ± 3
3	6	19 ± 4	880 ± 120
4	3	3.9 ± 0.6	4.6 ± 1.1
5	3	2.5 ± 0.5	7 ± 2
6	6	51 ± 18	200 ± 20

rins (**2** and **3**) as well as for porphyrin **6** (Table 2). Interestingly, the more highly charged sapphyrins **4** and **5** showed very low T/M and tumor to liver (T/L) ratios, in large measure because their content in muscle and liver tissue was significantly higher than that of the monocationic sapphyrins **1**−**3** and the porphyrin **6** (ca. 30 μg g^{-1} for muscle and 13−25 μg g^{-1} for liver, both on day 3 following standard administration). By contrast, sapphyrin **1**, a system with neutral side chains and a minimal level of water solubilizing substituents, displayed a rather high tumor content (215 μg g^{-1}), while exhibiting the best tumor specificity by virtue of its very low uptake/retention in all the normal tissues (content < 1.5 μg g^{-1} in all cases).

The D-glucosamine functionalized compounds **3** and **6** were both found in very low concentrations in the liver (<1.5 μg g^{-1}) but were found in relatively high concentrations in the spleen. Because of the extremely low liver content, these compounds had the highest T/L ratios; 884 for **3** and 198 for **6**. While the exact origins of the low liver content remain to be clarified, they could reflect a high rate of metabolism for these compounds, something that would be expected given the fact that they are functionalized with naturally occuring sugar moieties.

The poly(ethylene glycol) functionalized sapphyrin **2** was found in relatively high concentrations in the reticuloendothelial tissues (liver, kidney, and spleen), which take part in the transport of serum proteins. Kessel and others have reported that the distribution of certain porphyrins is directly related to the number of low-density lipoprotein (LDL) receptors in these tissues (liver > kidney > lung > spleen).[27−31] Tumor cells also have an elevated number of LDL receptors that can take part in the uptake of certain porphyrins. The biodistribution of sapphyrin **2** at day 6 shows a trend that matches the relative concentrations of LDL receptors; tumor > liver kidney > lung spleen (134, 61, 59, 11.1, 10.7 μg g^{-1}, respectively, following standard administration). This sapphyrin was also found to exhibit the highest blood content of the five sapphyrins in this study. While not a proof, these findings are consistent with a transport mechanism in which sapphyrin **2** binds to various LDLs in the blood, with this binding then serving to define in large measure the uptake, biolocalization, and clearance of this specific drug candidate.

Conclusion

Several novel water-soluble sapphyrins were prepared and characterized. Among these, the sapphyrins bearing neutral solubilizing groups (i.e., **1**−**3**) were found to have selectivities for tumor tissue over surrounding tissues that are as good or better than that seen for water soluble porphyrins, including a functionalized protoporphyrin XI control system (**6**).[4,5,8,9,32−34] The incorporation of charged moieties into the sapphyrin architecture (as in compounds **4** and **5**) significantly reduced the tumor localization, a finding that is thought to reflect in large measure the relatively high concentrations in normal tissues observed for these systems. The tetrahydroxy sapphyrin **1** exhibited the best tumor-to-muscle ratio, whereas the incorporation of glucosamides onto the sapphyrin (**3**) and porphyrin (**6**) cores afforded the best tumor-to-liver ratios, perhaps as the result of altering metabolic and clearance pathways. While further work will be required to map out further the specific determinants of biolocalization as they apply to sapphyrins, the present results certainly serve to highlight how changes in peripheral substitution patterns can be used to alter dramatically the biolocalization properties of a potential PDT sensitizer.

Experimental Section

General Methods. Proton and ^{13}C NMR spectra were obtained at 25 °C using a Varian Unity Plus spectrometer at 300 and 75 MHz, respectively. Mass spectrometric analyses were performed using a Finnigan MATTSQ 700 mass spectrometer. High-resolution mass spectra were obtained on a VGZAB2-E mass spectrometer. Elemental analyses were obtained by the Analytical Chemistry Department at the Institute for Chemical Technology, Prague.

Cancer Model. Outbreed female nude mice, strain CD-1, weighing 18−20 g, were used in the biolocalization experiments. The mice were obtained from AnLab Ltd. − Charles River, kept in laminal air flow boxes for small laboratory animals (KAT-F−SZ/1), provided with radiation-sterilized SAWO Research Bedding (AnLab Ltd. − Charles River), and fed with radiation-sterilized diet ST-1 (Bergman). They received autoclaved water ad libitum. Pieces of human pancreatic adenocarcinoma (line UB 290896), 2 × 2 mm in size, were transplanted subcutaneously on the right flanks of the nude mice. The animals were employed in biodistribution experiments when the tumor diameter reached 4.5−5.5 mm. 25 mice were used for each compound (5 animals per time point plus 5 as controls).

Biodistribution Assay. Aqueous solutions (∼1 mmol L^{-1}) of each macrocycle were injected subcutaneously into tumor-bearing mice (15 μmol kg^{-1} per day for 3 days). The animals were killed 1, 3, 6, and 9 days after the last injection. Tumor, liver, lung, kidney, blood, heart, spleen, brain, and muscle tissues were taken for analysis.

UV−Vis Absorption Spectroscopy. The unfrozen tissue samples (0.05−1 g) were homogenized with 5 mL of water-methanol mixture (1:4 v/v) for 30 s in a homogenizer. The homogenates were then transformed to test tubes, covered, stirred and left for 7 days at 8 °C, thus allowing for drug extraction. The samples were then centrifuged at 1000 g on a Hittich EBA 8S centrifuge. The supernatants were collected and subjected to UV−Vis spectral analysis. Spectra were recorded in quartz cuvettes of 1 cm path length using a diode array Hewlett-Packard 590A UV−Vis spectrophotometer. A water−methanol (1:4 v/v) mixture was used as a blank. Absorption measurements were made in the range of 190−900 nm, with a spectral bandwidth of 2 nm, and a data interval of 2 nm. With the assistance of the Hewlett-Packard UV software supplied with the spectrometer, differential spectra were calculated for tissue extracts obtained from injected mice vs noninjected mice, and used to estimate drug uptake. Calibration curves used to estimate photosensitizer content were made up using standard sapphyrin solutions (1×10^{-5} − 7×10^{-5} mg mL^{-1}) in the same water−methanol mixture that was used to effect the tissue extractions. The sapphyrin concentration in each tissue sample was then calculated from the intensity of the Soret band absorption maximum.

General Procedure for the Preparation of Sapphyrins 2−5. Five equivalents of amines **10**−**13** were added to a solution of the acid chloride sapphyrin **9** in dry dichloromethane. Catalytic amounts of (dimethylamino)pyridine and pyridine were then added. Immediately after these additions, the reaction vessel was covered in foil (to protect it from light) and stirred for 24 h under an argon blanket. The individual reaction mixtures were then evaporated to dryness, and the resulting protected sapphyrins purified by column chromatography over silica gel using mixtures of between 1 and 10% methanol in dichloromethane as the eluent. The acetate protected sapphyrins were dissolved in a methanolic solution of KOH (1 equiv. per acetate) and stirred for 4 h. The pH was then adjusted to 6 with hydrochloric acid and the reaction stripped of solvents. The deprotected polyhydroxy sapphyrins were then extracted from the resultant solid using a 3:1 dichloromethane/methanol mixture, stripped of solvents in vacuo, and recrystallized from a 1:3 ethanol/hexane mixture. The *t*-BOC protecting groups were removed by dissolving the protected sapphyrins in dichloromethane, adding trifluoroacetic acid (5% v/v), and stirring for 2 h. Following evaporation of solvent and acid from the sample, the deprotected sapphyrins were purified by recrystallization from a 1:3 ethanol/hexane mixture.

1,12,13,22-Tetraethyl-8,17-bis[2-(2-(2-acetoxyethoxy)-ethoxy)ethylamino-carbonylethyl]-2,7,18,23-tetramethylsapphyrin (14). In accord with the above general procedure, the reaction of the bis-acid chloride sapphyrin **9** (72 mg, 0.1 mmol) with **10** (96 mg, 0.5 mmol) in dichloromethane gave 81 mg (78%) of **14** as a dark green solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.83 (6H, t, CH_2CH_3), 2.04 (6H, t, CH_2CH_3), 2.27 (6H, s, $COCH_3$), 2.75 (4H, t, NCH_2), 2.77 (4H, t, OCH_2), 2.80 (4H, t, OCH_2), 2.98 (4H, t, OCH_2), 3.06 (4H, t, CH_2CH_2CON), 3.14 (4H, t, OCH_2), 3.20 (4H, t, OCH_2), 3.61 (6H, s, CH_3), 4.00 (6H, s, CH_3), 4.26 (4H, q, CH_2CH_3), 5.02 (4H, q, CH_2CH_3), 5.31 (4H, t, CH_2CH_2CON), 10.33 (4H, s, *meso*-H), 10.66 (4H, s, *meso*-H). ^{13}C NMR (75 MHz, CD_3OD): δ 13.15, 15.90, 18.21, 19.17, 19.55, 21.03, 22.25, 24.04, 39.41, 40.33, 55.62, 67.90, 69.09, 70.99, 89.52, 91.26, 97.02, 120.63, 128.62, 130.80, 133.35, 135.91, 138.56, 139.19, 142.48, 144.15, 171.21, 173.75. FAB HRMS calcd for $C_{58}H_{80}N_7O_{10}$ ($[M + H]^+$) 1034.59667; obsd 1034.59698.

1,12,13,22-Tetraethyl-8,17-bis[2-(2-(2-hydroxyethoxy)-ethoxy)ethylamino-carbonylethyl]-2,7,18,23-tetramethylsapphyrin (2). After subjecting the acetylated derivative **14** (19.6 mg, 0.0190 mmol) to saponification as described in the general procedures, 15.3 mg (85%) of **2** was isolated as a lustrous green solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.85 (6H, t, CH_2CH_3), 2.01 (6H, t, CH_2CH_3), 2.72 (4H, t, NCH_2), 2.76 (4H, t, OCH_2), 2.82 (4H, t, OCH_2), 2.90 (4H, t, OCH_2), 3.05 (4H, t, CH_2CH_2CON), 3.14 (4H, t, OCH_2), 3.19 (4H, t, OCH_2), 3.61 (6H, s, CH_3), 4.04 (6H, s, CH_3), 4.27 (4H, q, CH_2CH_3), 5.03 (4H, q, CH_2CH_3), 5.29 (4H, t, CH_2CH_2CON), 10.34 (4H, s, *meso*-H), 10.61 (4H, s, *meso*-H). ^{13}C NMR (75 MHz, CD_3OD): δ 13.12, 15.96, 18.22, 19.14, 19.56, 21.07, 22.25, 23.98, 39.41, 40.29, 55.66, 67.87, 69.08, 70.97, 89.54, 91.31, 96.78, 120.70, 128.69, 130.83, 133.31, 135.91, 138.65, 139.15, 142.48, 144.09, 173.75. FAB HRMS calcd for $C_{54}H_{76}N_7O_8$ ($[M + H]^+$) 950.57554, obsd 950.57492. Anal. ($C_{54}H_{75}N_7O_8$) calc. C 68.26, H 7.96, N 10.32; found C 68.04, H 8.13, N 10.09.

1,12,13,22-Tetraethyl-8,17-bis[(1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-α,β-D-glucopyranose)-carbonylethyl]-2,7,-18,23-tetramethylsapphyrin (15). Using the standard general procedure, reaction of the bis-acid chloride sapphyrin **9** (69 mg, 0.1 mmol) with **11** (174 mg, 0.5 mmol) in dichloromethane gave 115 mg (85%) of **15** as a dark green solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ −4.91 (2H, s, NH), −4.59 (1H, s, NH), −4.30, 1.94−2.02 (24H, m, acetyl), 2.06 (6H, t, CH_2CH_3), 2.12−2.27 (6H, t, CH_2CH_3), 3.41−4.72 (10H, m, glucose moiety), 4.05 (6H, s, CH_3), 4.22 (6H, s, CH_3), 4.54 (4H, q, CH_2CH_3), 4.63 (4H, q, CH_2CH_3), 5.04 (2H, m, β glucose H), 5.22 (2H, d, α glucose H), 8.15 amide (m, 2H, amide), 11.59 (2H, s, *meso*-H), 11.69 (2H, s, *meso*-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.92, 15.60, 17.61, 20.56, 20.93, 21.16, 24.27, 32.22, 62.00. 64.28, 68.25, 91.50, 91.59, 98.51, 102.63, 129.75, 130.40, 132.57, 135.19, 135.63, 137.82, 138.45, 142.95, 169.50, 170.36, 171.32. FAB HRMS calcd for $C_{70}H_{89}N_7O_{20}$ ($[M + 2H]^+$) 1347.61428; obsd 1347.61624.

1,12,13,22-Tetraethyl-8,17-bis[(2-amino-2-deoxy-α,β-D-glucopyranose)-carbonylethyl]-2,7,18,23-tetramethylsapphyrin (3). From 13.4 mg (0.0099 mmol) of the acetylated derivative **15**, 10.4 mg (88%) of **3** was isolated as a lustrous green solid. ^{1}H NMR (300 MHz, $CDCl_3$ with 25% CD_3OD): δ 2.08 (6H, t, CH_2CH_3), 2.18 (6H, t, CH_2CH_3), 3.46 (4H, t, CH_2CH_2CON), 3.91 (6H, s, CH_3), 4.05−4.44 (8H, m, glucose CH), 4.11 (6H, s, CH_3), 4.39 (4H, q, CH_2CH_3), 4.57 (4H, s, CH_2CH_3), 5.01 (4H, t, CH_2CH_2CON), 11.12 (2H, s, *meso*-H), 11.19 (2H, s, *meso*-H). ^{13}C NMR (75 MHz, $CDCl_3$ with 25% CD_3OD): δ 12.56, 15.79, 16.12, 16.59, 17.66, 18.76, 20.59, 23.33, 35.60, 36.49, 37.12, 52.63, 59.73, 60.77, 75.76, 90.17, 90.91, 97.84, 128.17, 130.58, 135.34, 136.47, 137.13, 142.15, 171.68. FAB HRMS calcd for $C_{54}H_{72}N_7O_{12}$ ($[M + H]^+$) 1010.52390; obsd 1010.52334. Anal. ($C_{54}H_{71}N_7O_{12}$) calc C 64.20, H 7.08, N 9.71; found C 64.01, H 7.22, N 9.49.

1,12,13,22-Tetraethyl-8,17-bis[2-(2-(2-(N-*tert*-butyloxy-carbonyl)aminoethoxy)ethoxy)ethylamino-carbonylethyl]-2,7,18,23-tetramethylsapphyrin (16). Standard reaction of the bis-acid chloride sapphyrin **9** (72 mg, 0.1 mmol) with **12** (124 mg, 0.5 mmol) in dichloromethane, followed by purification in the usual way, gave 94 mg (82%) of **16** as a dark green solid. ^{1}H NMR (300 MHz, 5% CD_3OD in $CDCl_3$): δ 1.18 (18H, s, $C(CH_3)_3$), 1.99 (6H, t, CH_2CH_3), 2.15 (6H, t, CH_2CH_3), 2.50 (4H, t, NCH_2), 2.68 (4H, t, NCH_2), 3.02 (4H, t, OCH_2), 3.13 (4H, t, OCH_2), 3.22 (4H, t, OCH_2), 3.35 (4H, t, CH_2CH_2CON), 3.54 (4H, t, OCH_2), 3.94 (6H, s, CH_3), 4.07 (6H, s, CH_3), 4.34 (4H, q, CH_2CH_3), 4.55 (4H, q, CH_2CH_3), 4.91 (4H, t, CH_2CH_2CON), 11.02 (4H, s, *meso*-H). ^{13}C NMR (75 MHz, 5% CD_3OD in $CDCl_3$): δ 12.44, 16.06, 17.77, 18.49, 20.46, 20.59, 23.38, 27.97, 28.22, 38.89, 39.36, 69.06, 69.23, 69.33, 69.99, 70.07, 79.04, 90.41, 127.34, 133.66, 133.91, 135.20, 135.80, 136.36, 137.06, 137.79, 142.69, 142.82, 155.89, 172.90. FAB HRMS calcd for $C_{64}H_{94}N_9O_{10}$ ($[M + H]^+$) 1148.712366; obsd 1148.711245.

1,12,13,22-Tetraethyl-8,17-bis[2-(2-(2-aminoethoxy)eth-oxy)ethylamino-carbonylethyl]-2,7,18,23-tetramethylsapphyrin (4). From 17.7 mg (0.0154 mmol) of the *t*-BOC protected derivative **16**, 13.2 mg (90%) of **4** was isolated as a lustrous green solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.87 (6H, t, CH_2CH_3), 2.05 (6H, t, CH_2CH_3), 2.44 (4H, t, NCH_2), 2.66 (4H, t, NCH_2), 2.73 (4H, t, OCH_2), 2.83 (4H, t, OCH_2), 2.92 (4H, t, OCH_2), 3.05 (4H, t, CH_2CH_2CON), 3.16 (4H, t, OCH_2), 3.59 (6H, s, CH_3), 4.02 (6H, s, CH_3), 4.29 (4H, q, CH_2CH_3), 5.07 (4H, q, CH_2CH_3), 5.33 (4H, t, CH_2CH_2CON), 10.43 (4H, s, *meso*-H), 10.69 (4H, s, *meso*-H). ^{13}C NMR (75 MHz, 5% CD_3OD in $CDCl_3$): δ 13.54, 16.34, 18.05, 18.37, 19.31, 21.90, 22.04, 24.58, 39.43, 40.00, 40.18, 58.31, 67.21, 69.90, 70.63, 91.08, 97.62, 127.31, 128.34, 128.70, 131,54, 133.69, 134.17, 138.83, 142.31, 143.97, 174.53. FAB HRMS calcd for $C_{54}H_{78}N_9O_6$ ($[M + H]^+$) 948.607507; obsd 948.605638. Anal. ($C_{54}H_{77}N_9O_6$) calc C 68.40, H 8.18, N 13.29; C 68.25, H 8.37, N 13.06.

1,12,13,22-Tetraethyl-8,17-bis[2-bis(2-(N-*tert*-butyloxy-carbonyl)aminoethyl)amino)ethyl)amino-carbonylethyl]-2,7,18,23-tetramethylsapphyrin (17). The reaction of the bis-acid chloride sapphyrin **9** (71 mg, 0.1 mmol) with **13** (173 mg, 0.5 mmol) in dichloromethane gave 107 mg (80%) of **17** as a dark green solid. ^{1}H NMR (300 MHz, 5% CD_3OD in $CDCl_3$): δ 1.05 (36H, s, $C(CH_3)_3$), 1.97 (8H, t, NCH_2), 2.14 (6H, t, CH_2CH_3), 2.44 (6H, t, CH_2CH_3), 2.86 (8H, t, NCH_2), 3.01 (4H, t, NCH_2), 3.08 (4H, t, NCH_2), 3.38 (4H, t, CH_2CH_2CON), 3.94 (6H, s, CH_3), 4.08 (6H, s, CH_3), 4.34 (4H, q, CH_2CH_3), 4.56 (4H, q, CH_2CH_3), 4.93 (4H, t, CH_2CH_2CON), 11.01 (2H, s, *meso*-H), 11.08 (2H, s, *meso*-H). ^{13}C NMR (75 MHz, 5% CD_3OD in $CDCl_3$): δ 12.41, 16.00, 17.72, 18.46, 20.49, 23.41, 27.82, 28.00, 37.38, 37.98, 38.51, 39.09, 53.33, 54.04, 90.46, 90.62, 97.00, 97.81, 127.41, 133.44, 133.77, 135.09, 135.85, 137.48, 137.64, 142.85, 156.30, 173.16. FAB HRMS calcd for $C_{74}H_{114}N_{13}O_{10}$ ($[M + H]^+$) 1344.881163; obsd 1344.880515.

1,12,13,22-Tetraethyl-8,17-bis[2-bis(2-aminoethyl)amino)ethyl)amino-carbonylethyl]-2,7,18,23-tetramethylsapphyrin (5). Using the generalized deprotection strategy and 21.2 mg (0.0158 mmol) of the *t*-BOC protected derivative **17**, 12.2 mg (82%) of **5** was isolated as a lustrous green solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.59 (8H, t, NCH_2), 2.40 (6H, t, CH_2CH_3), 2.65 (6H, t, CH_2CH_3), 3.40 (4H, t, NCH_2), 3.44 (8H, t, NCH_2), 3.58 (4H, t, NCH_2), 3.72 (6H, s, CH_3), 3.75 (6H, s, CH_3), 3.89 (4H, t, CH_2CON), 4.09 (4H, q, CH_2CH_3), 4.54 (4H, q, CH_2CH_3), 4.83 (4H, t, CH_2CH_2CON), 10.87 (2H, s, *meso*-H), 11.22 (2H, s, *meso*-H). ^{13}C NMR (75 MHz, CD_3OD): δ 13.55, 16.23, 17.95, 18.37, 19.53, 21.87, 22.15, 24.29, 35.40, 39.44, 51.63, 54.61, 91.23, 97.77, 97.83, 120.47, 128.37, 130.34, 133.69, 135.61, 138.89, 139.12, 142.31, 144.11, 175.59. FAB HRMS calcd for $C_{54}H_{82}N_{13}O_2$ ($[M + H]^+$) 944.671445; obsd 944.669615. Anal. ($C_{54}H_{81}N_{13}O_2$) calc C 68.68, H 8.65, N 19.28; C 68.36, H 8.78, N 18.90.

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References

(1) Pandey, R. K. Recent Advances in Photodynamic Therapy. *J. Porphyrins Phthalocyanines* **2000**, *4*, 368−373.
(2) Mody, T. D. Parmaceutical Development and Medical Applications of Porphyrin-type Macrocycles. *J. Porphyrins Phthalocyanines* **2000**, *4*, 362−367.
(3) Pandey, R. K.; Zheng, G. Porphyrins as Photosensitizers in Photodynamic Therapy. *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: San Diego, 2000; Vol 6.
(4) Dougherty, T. J. Photosensitizers: Therapy and Detection of Malignant Tumors. *Photochem. Photobiol.* **1987**, *45*, 879−889.
(5) Bellnier, D. A.; Ho, Y. K.; Pandey, R. K.; Missert, J. R.; Dougherty, T. J. Distribution and Elimination of Photofrin II in Mice. *Photochem. Photobiol.* **1989**, *50*, 221−228.
(6) Jasat, A.; Dolphin, D. Expanded Porphyrins and Their Heterologs. *Chem. Rev.* **1997**, *97*, 2267−2340.
(7) Sessler, J. L.; Weghorn, S. J. *Expanded, Contracted and Isomeric Porphyrins*; Elsevier: Oxford, 1997.
(8) Blant, S. A.; Grosjean, P.; Ballini, J.-P.; Wagnieres, G.; van den Bergh, H.; Fontolliet, C.; Monnier, P. Localization of Tetra(m-hydroxyphenyl)chlorin (Foscan) in Human Healthy Tissues and Squamous Cell Carcinomas of the Upper Aero-digestive Tract, the Esophagus and the Bronchi: a Fluorescence Microscopy Study. *J. Photochem. Photobiol. B: Biol.* **2001**, *61*, 1−9.
(9) Kessel, D.; Smith, K. M.; Pandey, R. K.; Shiau, F.-Y.; Henderson, B. Photosensitization with Bacteriochlorins. *Photochem. Photobiol.* **1993**, *58*, 200−203.
(10) Pandey, R. K.; Tsuchida, T.; Constantine, S.; Zheng, G.; Medforth, C.; Kozyrev, A.; Mohammad, A.; Rodgers, M. A. J.; Smith, K. M.; Dougherty, T. J. Synthesis, photophysical properties and in vivo photosensitizing activity of some novel bacteriochlorins. *J. Med. Chem.* **1997**, *40*, 3770−3779.
(11) Young, S. W.; Woodburn, K. W.; Wright, M.; Mody, T. D.; Fan, Q.; Sessler, J. L.; Dow, W. C.; Miller, R. A. Lutetium Texaphyrin (PCI-0123): a Near-Infrared, Water-Soluble Photosensitizer. *Photochem. Photobiol.* **1996**, *63*, 892−897.
(12) Miller, R. A.; Woodburn, K.; Fan, Q.; Renschler, M. F.; Sessler, J. L.; Koutcher, J. A. In Vivo Animal Studies With Gadolinium (III) Texaphyrin as a Radiation Enhancer. *Int. J. Radiat. Oncol. Biol. Phys.* **1999**, *45*, 981−989.
(13) Maiya, B. G.; Cyr, M.; Harriman, A.; Sessler, J. L. In Vitro Photodynamic Activity of Diprotonated Sapphyrin: a 22-Pi-Electron Pentapyrrolic Porphyrin-like Macrocycle. *J. Phys. Chem.* **1990**, *94*, 3597−3601.
(14) Magda, D.; Wright, M.; Miller, R. A.; Sessler, J. L.; Sansom, P. I. Sequence-Specific Photocleavage of DNA by an Expanded Porphyrin with Irradiation Above 700 nm. *J. Am. Chem. Soc.* **1995**, *117*, 3629−3630.
(15) Sessler, J. L.; Andrievsky, A.; Sansom, P. I.; Král, V.; Iverson, B. L. Enhanced DNA Photocleavage and Binding Properties of Sapphyrin-Polyamine Conjugates. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1433−1436.
(16) Sessler, J. L.; Sansom, P. I.; Král, V.; O'Connor, D.; Iverson, B. L. Sapphyrin-Oligonucleotide Conjugates. Novel Sequence-Specific DNA Photomodifying Agents with Increased Binding Affinity. *J. Am. Chem. Soc.* **1996**, *118*, 12322−12330.
(17) Woodward, R. B. In *Special Publication no. 21*; The Chemical Society: London, 1966.
(18) Bauer, V. J.; Clive, D. L. J.; Dolphin, D.; Paine, J. B., III; Harris, F. L.; King, M. M.; Loder, J.; Wang, S. W. C.; Woodward, R. B. Sapphyrins: Novel Aromatic Pentapyrrolic Macrocycles. *J. Am. Chem. Soc.* **1983**, *105*, 6429−6436.
(19) Král, V.; Furuta, H.; Shreder, K.; Lynch, V.; Sessler, J. L. Protonated Sapphyrins. Highly Effective Phosphate Receptors. *J. Am. Chem. Soc.* **1996**, *118*, 1595−1607.
(20) Iverson, B. L.; Shreder, K.; Král, V.; Sessler, J. L. Phosphate Recognition by Sapphyrin. A New Approach to DNA Binding. *J. Am. Chem. Soc.* **1993**, *115*, 11022−11023.
(21) Iverson, B. L.; Shreder, K.; Král, V.; Sansom, P.; Lynch, V.; Sessler, J. L. Interaction of Sapphyrin with Phosphorylated Species of Biological Interest. *J. Am. Chem. Soc.* **1996**, *118*, 1608−1616.
(22) Parmeswaran, D.; Ganesan, S.; Thanigaivelu, K.; Pushpan, S.; Srinivasan, A.; Kumar, M. R.; Chandrasekar, T. K. In *SPIE Conference on Potical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy VIII*; SPIE: San Jose, California, 1999; Vol. 3592, pp 94−100.
(23) Synytsya, A.; Král, V.; Poucková, P.; Volka, K. Resonance Raman and UV−Visible Spectroscopic Studies of Water-Soluble Sapphyrin Derivative: Drug Localization in Tumor and Normal Mice Tissues. *Appl. Spectrosc.* **2001**, *55*, 142−148.
(24) Sessler, J. L.; Brucker, E. A. The First "Crowned" Expanded Porphyrin. *Tetrahedron Lett.* **1995**, *36*, 1175−1176.
(25) Král, V.; Sessler, J. L. Molecular Recognition via Base-Pairing and Phosphate Chelation. Ditopic and Tritopic Sapphyrin-Based Receptors for the Recognition and Transport of Nucleotide Monophosphates. *Tetrahedron* **1995**, *51*, 539−554.
(26) Fuhrhop, J. H.; Demoulin, C.; Boettcher, C.; Koening, J.; Siggel, U. Chiral Micellar Porphyrin Fibers With 2-Aminoglycosamide Headgroups. *J. Am. Chem. Soc.* **1992**, *114*, 4159−4165.
(27) Kessel, D. Porphyrin-Lipoprotein Association as a Factor in Porphyrin Localization. *Cancer Lett.* **1986**, *33*, 183−188.
(28) Nakajima, S.; Takemura, T.; Sakata, I. Tumor-Localizing Activity of Porphyrin and its Affinity to LDL, Transferrin. *Cancer Lett.* **1995**, *92*, 113−118.
(29) Jori, G.; Reddi, E. The Role of Lipoproteins in the Delivery of Tumor-Targeting Photosensitizers. *Int. J. Biochem.* **1993**, *25*, 1369−1375.
(30) De Smidt, P. C.; Versluis, A. J.; Van Berkel, T. J. C. Properties of Incorporation, Redistribution, and Integrity of Porphyrin Low-Density Lipoprotein Complexes. *Biochemistry* **1993**, *32*, 2916−2922.
(31) Maziere, J. C.; Morliere, P.; Santus, R. The Role of the Low-Density Lipoprotein Receptor Pathway in the Delivery of Lipophilic Photosensitizers in the Photodynamic Therapy of Tumors. *J. Photochem. Photobiol., B* **1991**, *8*, 351−360.
(32) Moan, J.; Peng, Q.; Evensen, J. F.; Berg, K.; Western, A.; Rimington, C. Photosensitizing Efficiencies, Tumor- and Cellular Uptake of Different Photosensitizing Drugs Relevant for Photodynamic Therapy of Cancer. *Photochem. Photobiol.* **1987**, *46*, 713−721.
(33) Tronconi, M.; Colombo, A.; De Cesare, M.; Marchesini, R.; Woodburn, K. W.; Reiss, J. A.; Phillips, D. R.; Zunino, F. Biodistribution of Hematoporphyrin Analogues in a Lung Carcinoma Model. *Cancer Lett.* **1995**, *88*, 41−48.
(34) Zheng, G.; Potter, W. R.; Camacho, S. H.; Missert, J. R.; Wang, G.; Bellnier, D. A.; Henderson, B. W.; Rodgers, M. A. J.; Dougherty, T. J.; Pandey, R. K. Synthesis, Photophysical Properties, Tumor Uptake, and Preliminary in Vivo Photosensitizing Efficacy of a Homologous Series of 3-(1′-Alkyloxy)ethyl-3-devinylpurpurin-18-N-alkylimides with Variable Lipophilicity. *J. Med. Chem.* **2001**, *44*, 1540−1559.

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