Water-Soluble, Core-Modified Porphyrins as Novel, Longer-Wavelength-Absorbing Sensitizers for Photodynamic Therapy. II. Effects of Core Heteroatoms and *Meso*-Substituents on Biological Activity

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Water-soluble, core-modified porphyrins were prepared and evaluated as sensitizers for photodynamic therapy (PDT). The addition of an aromatic aldehyde to 2,5-dilithiothiophene or -selenophene gave diol **3** as a nearly equimolar mixture of meso and d,l diastereomers, which gave a single diastereomer following careful recrystallization. The condensation of pyrrole with a diol **3** using catalytic BF_3-etherate gave bispyrrolochalcogenophenes (**4**). Condensation of a diol **3** with **4** in the presence BF_3-etherate gave 21,23-dichalcogenaporphyrins (**5**). 21-Thiaporphyrins (**6**) were prepared by condensation of a diol **3** with excess pyrrole and benzaldehyde in the presence of tetrachlorobenzoquinone and catalytic BF_3-etherate. Sulfonation of **5** and **6** with concentrated sulfuric acid at 100 °C gave sulfonated derivatives **7**−**15**. Bis-4-methoxy-21,23-dithiaporphyrins **5h** and **5l** were demethylated with BBr_3, and the resulting phenols were alkylated with ethyl bromoacetate. Saponification gave 21,23-dithiaporphyrin dicarboxylate salts **16** and **17**. The 21,23-core-modified porphyrins gave band I absorption maxima (λ_{max} of 689−717 nm) at longer wavelengths than band I for the corresponding 21-core-modified porphyrins, but both classes had band I maxima at longer wavelengths than either $TPPS_4$ or Photofrin (λ_{max} of 630 nm for both). The core heteroatoms had little effect on either absorption maxima or quantum yields of singlet oxygen generation in **7**−**17**. The meso substituents had a greater impact on absorption maxima. Compounds **7**−**17** were evaluated for phototoxicity against Colo-26 cells in culture using 4 J cm^{-2} of 570−800 nm light. Compounds **8**−**12**, **14**, **16**, and **17** gave a 50% cell kill in vitro at a lower concentration than Photofrin [5.7 mg (9 μmol)/kg]. Compounds **14**, **16**, and **17** gave a 50% cell kill with 4 J cm^{-2} of light and submicromolar concentrations of sensitizer. Sensitizers **8** and **11** showed no toxicity or side effects in BALB/c mice observed for 90 days following a single intravenous injection of 10 mg/kg of sensitizer. Distribution studies show that sensitizer **8** accumulates in the tumors of BALB/c mice. PDT with **8** at 0.125 mg (0.13 μmol)/kg or **11** at 2.5 mg (2.5 μmol)/kg and 135 J cm^{-2} of 694 nm light was comparable to PDT with Photofrin at 2.5 mg (4 μmol)/kg and 135 J cm^{-2} of 630 nm light against Colo-26 tumors in BALB/c mice.

Introduction

In the treatment of cancer, one protocol that can better differentiate cancerous and normal tissue is photodynamic therapy (PDT).[1,2] PDT combines light and endogenous oxygen with a photosensitizer localized in or around the tumor. Irradiation of the sensitizer produces a cascade of biochemical events that inactivate cancer cells either directly through attack at specific cellular sites or indirectly through the induction of vascular damage to blood vessels feeding the tumor.[1,2] PDT has regulatory approval in the USA, Canada, The Netherlands, France, Germany, and Japan for cancers of the lung, digestive tract, and genitourinary tract using Photofrin as a photosensitizer.[1,3−5] PDT with Photofrin is also being evaluated as a protocol for treating cancers of the head and neck region[6] and for treating pancreatic cancer[7] as well as a possible therapy against Karposi's sarcoma and cancers of the brain, breast (both primary and metastatic), skin, and abdomen.[8]

Although Photofrin has been shown to be effective against a number of malignancies, it is not the "ideal" photosensitizer. Photofrin and most other porphyrin-related sensitizers have a weak absorbance in the red region of the spectrum (≥630 nm where the penetration of light in tissue is optimal) and induce long-lasting skin photosensitivity (2−3 months) due to the retention of porphyrin moieties in cutaneous tissue.[9] Because Photofrin is not a well-defined, single agent,[10] it is difficult to modify it chemically for the investigation of structure−activity relationships. These limitations have

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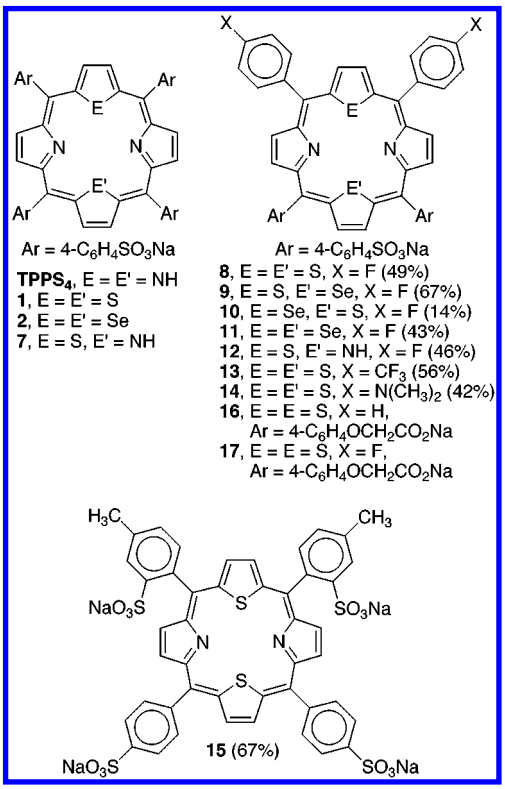
Chart 1



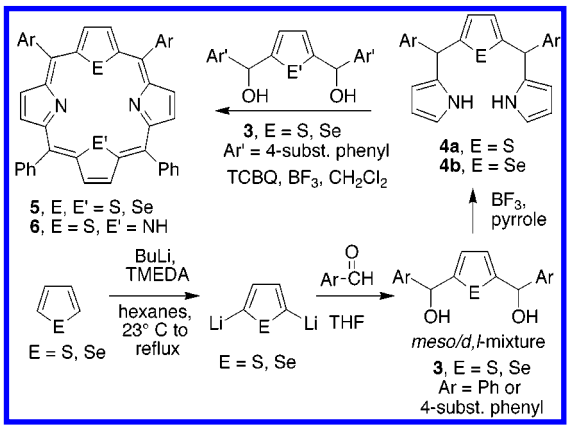
stimulated research efforts toward the development of second and third generation sensitizers for PDT that better fit the definition of an ideal sensitizer.[8]

We have recently described the synthesis and properties of 21,23-dithia (**1**) and 21,23-diselena (**2**) core-modified derivatives[11] of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin ($TPPS_4$, Chart 1). $TPPS_4$ once was a promising photosensitizer displaying excellent membrane permeability and lysosomal accumulation in cells and is one of the more selective porphyrins for accumulation in tumors with demonstrated efficacy both in vitro and in vivo.[12] The development of $TPPS_4$ as a potential photosensitizer in the clinic has been hindered by its reported neurotoxicity in mice at high concentrations.[13]

Structurally, the chalcogen heteroatoms of the 21,23-core-modified porphyrins fill the core and prevent binding to metals,[14] which is a striking difference from the normal porphyrin core and which might be expected to impart different biological properties to core-modified porphyrins in comparison to the normal tetrapyrrolic porphyrins. The 21,23-dithiaporphyrin **1** was found to be more potent than $TPPS_4$ both in vitro and in vivo although compound **1** generates singlet oxygen less efficiently than $TPPS_4$.[11] Both **1** and **2** absorb light of significantly longer wavelengths (λ_{max} of 695 nm for **1** and **2** in water) than either $TPPS_4$ or Photofrin (both with λ_{max} of 630 nm in water).[11]

Sulfonated 21-thia and 21-selena core-modified porphyrins have been prepared and evaluated as sensitizers for PDT. The 21-thia and 21-selena derivatives absorb light of longer wavelengths than either $TPPS_4$ or Photofrin.[15] Sulfonated 21-core-modified porphyrins were reported to be comparable to chlorin e6 for efficacy in vivo with BFS1 sarcoma-bearing mice.[16] Furthermore, the 21-selena analogue showed no skin photosensitization in animals irradiated 24 h postinjection.[16]

Scheme 1



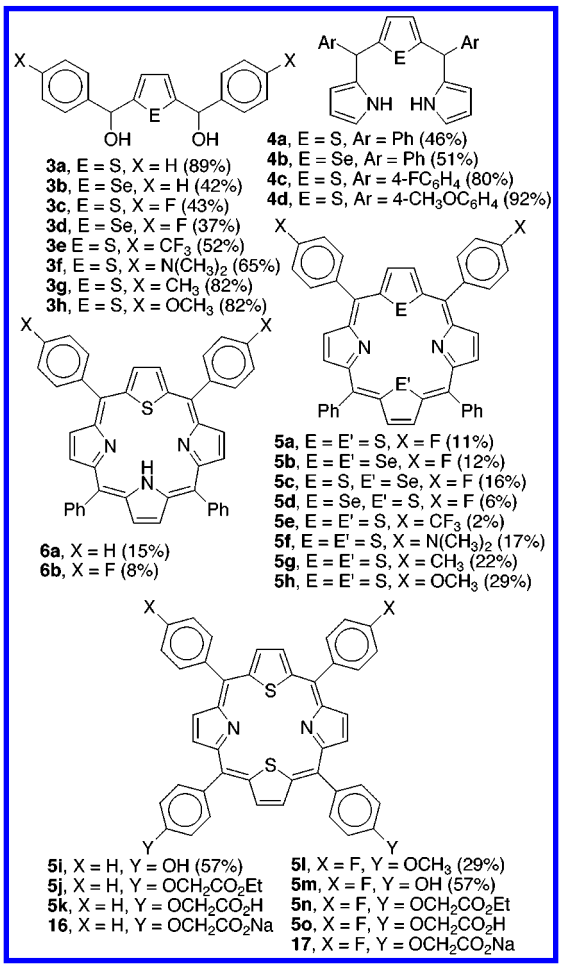
In view of our promising results with **1**[11] and the reported results with the 21-core-modified porphyrins,[16] we were interested in examining 21- and 21,23-core-modified porphyrins more thoroughly as sensitizers for PDT. Herein, we describe a general synthetic strategy to 21- and 21,23-core-modified porphyrins that permits the regiospecific incorporation of S, Se, and N−H groups in the 21- and 23-positions and the incorporation of different pairs of meso substituents at the 5- and 10-positions or the 15- and 20-positions. We also describe the effects of heteroatom substitution and meso group substitution on physical and photophysical properties and on biological properties of these new core-modified porphyrins.

Chemistry

Synthesis. A. Water-Soluble Sulfonated Derivatives. Analogues of $TPPS_4$ have been prepared and a limited quantitative structure−activity relationship (QSAR) has been developed,[12b,c,17] in which the number and placement of solubilizing groups markedly affect biodistribution, localization, and efficacy. In the series of porphyrin analogues of $TPPS_4$, molecules with two identical sulfonatoaryl groups at the 5- and 10-positions of the porphyrin core[12b,c,17] and with other substituents at the 15- and 20-positions have optimal properties for uptake and distribution. Our general synthetic approach to 21- and 21,23-core-modified porphyrins is shown in Scheme 1 and allows the various combinations of heteroatoms to be placed regiospecifically. The synthetic route also allows the 5- and 10-positions to be identical to or different from the 15- and 20-positions.

The 2,5-dilithiation of thiophene or selenophene was accomplished with excess BuLi−TMEDA (*N*,*N*,*N*,*N*-tetramethylethylenediamine) at −78 °C in hexanes.[14a] The addition of various aromatic aldehydes to the 2,5-dilithiochalcogenophenes gave the 2,5-bis(arylhydroxymethyl)chalcogenophenes (**3**) shown in Chart 2 in 37−89% isolated yield as a single, crystalline diastereomer (by ^1H and ^{13}C nuclear magnetic resonance (NMR)) following recrystallization of a mixture of meso and d,l diastereomers.[11] The condensation of pyrrole with 2,5-bis(arylhydroxymethyl)chalcogenophenes **3a**−**c** and **3h** was catalyzed by BF_3-etherate to give bis(arylpyrrolomethyl)chalcogenophenes **4a**−**d** in 46−92% isolated yields (Chart 2).

Chart 2



As we previously described for the preparation of **3a**,**b**,[11] the diols **3** can exist as either meso or d,l diastereomers, which should be comparable energetically and spectroscopically. The meso and d,l forms can interconvert in the presence of trace amounts of acid.[11] The crude diol **3** displayed ^{1}H NMR signals consistent with both diastereomers. However, slow recrystallization of the crude diol **3** gave crystalline materials that were isolated as a single diastereomer, although we cannot assign the meso or d,l stereochemistry with certainty. For **3c** and **3h**, the reported literature melting points are 103−104 and 154 °C,[14a] respectively, which are significantly different than the melting points of 77−79 °C for **3c** and 125−127 °C for **3h** that we observe. By ^{1}H and ^{13}C NMR, compounds **3c** and **3h** that we have isolated each appear to be a single, pure diastereomer, which may be the alternate diastereomer to those reported previously.[14a]

Compounds **3** and **4** are the building blocks for the regiospecific preparation of 21,23-core-modified porphyrins (**5**) and 21-core-modified porphyrins (**6**). Equimolar amounts of **3** and **4** in dichloromethane were condensed with catalytic BF_3-etherate in the presence of tetrachlorobenzoquinone (TCBQ)[15] to give 21,23-core-modified porphyrins (**5**) in isolated yields of 2−29% (Chart 2). Diols **3a** and **3c** were each condensed with pyrrole and benzaldehyde in the presence of TCBQ using BF_3-etherate as a catalyst[15] to give 21-thiaporphyrins **6a**[18] and **6b** in 15 and 8% yields, respectively (Chart 2).

Compounds **5** and **6** were sulfonated with concentrated sulfuric acid at 100 °C[11] to give the 4-sulfonatophenyl-substituted derivatives **7**−**14** in 14−56% yields (Chart 1). The core-modified porphyrins were isolated as the disodium salts following neutralization of the sulfuric acid with NaOH and reverse phase chromatography. Under the reaction conditions for the preparation of **14**, the dimethylanilino group of **5f** is protonated, thus deactivating the aniline ring to sulfonation. The deactivating fluoro and trifluoromethyl groups allow selective sulfonation of the phenyl substituents of **5a**−**e**, respectively.

Sulfonation of **5g** gave a tetrasulfonated product. The tolyl and phenyl groups of **5g** are close enough in reactivity that sulfonation gave a single tetrasulfonated material, which we have assigned structure **15** in Chart 1. MALDI time-of-flight (TOF) mass spectrometry confirmed the tetrasulfonation product (*m*/*z* 1085 for M + 1) and the 20 signals observed in the ^{13}C NMR spectrum are consistent with sulfonation of the phenyl rings at the 4-positions and monosulfonation of each tolyl group at either the 2-position or the 3-position (21 signals expected for either).

Dithiaporphyrin **5h** was unstable to the sulfonation conditions. No methoxy singlets were observed in the ^{1}H NMR spectrum of the product mixture following workup. Sulfonation of **5h** was not investigated further.

B. Water-Soluble Carboxylate Derivatives. Compounds **8**−**14** each have two sulfonato substituents to provide water solubility. For comparison purposes, we prepared the biscarboxylates **16** and **17**, whose pK_a values should be nearer physiological pH. Compound **16** was prepared via initial demethylation of dimethoxy porphyrin **5h** with BBr_3 to give bisphenol **5i** in 57% yield, which was alkylated with ethyl bromoacetate to give **5j** in 50% isolated yield. Saponification gave biscarboxylic acid **5k** in 91% yield, which was then converted to the disodium salt **16** in 70% yield.

Biscarboxylate **17** was prepared from porphyrin **5l** via a similar route. The condensation of pyrrole with either 2,5-bis(4-fluorophenylhydroxymethyl)thiophene (**3c**) or 2,5-bis(4-methoxyphenylhydroxymethyl)thiophene (**3h**) was catalyzed by BF_3-etherate in the presence of TCBQ[15] to give bis(4-fluorophenylpyrrolomethyl)thiophene (**4c**) in 80% isolated yield or bis(4-methoxyphenylpyrrolomethyl)thiophene (**4d**) in 92% isolated yield, respectively (Chart 2). Equimolar amounts of **3h** and **4c** in dichloromethane were condensed with BF_3-etherate in the presence of TCBQ[15] to give dithiaporphyrin **5l** (Chart 2) in 29% yield. Demethylation with BBr_3 gave bisphenol **5m** in 57% isolated yield. The bisphenol **5m** was treated with ethyl bromoacetate and sodium carbonate to give diester **5n** in 76% yield. The diester **5n** was saponified to the corresponding acid with NaOH in aqueous tetrahydrofuran (THF) to give carboxylic acid **5o** in 93% yield, which was then converted to the sodium salt **17** in 91% yield.

Characterization of Water-Soluble, Core-Modified Porphyrins. All of the sulfonated materials **7**−**15** and the dicarboxylates **16** and **17** gave parent ions by mass spectrometry [fast atom bombardment (FAB(+)), MALDI TOF, or Q-TOF electrospray] corresponding to the di- or tetrasodium salts. The elemental analyses in this series were consistent with several waters of

crystallization per sulfonate or carboxylate group. In compounds **7**−**14**, sulfonation at the 4-position was indicated by the appearance of a new AA′BB′ pattern in the ^{1}H NMR spectrum and appropriate symmetry in the ^{13}C NMR spectrum for each sample.

From the ^{13}C NMR spectrum of **15**, the tolyl groups are sulfonated either at the 2-position adjacent to the dithiaporphyrin ring or at the 3-position adjacent to the methyl substituents. The ^{1}H NMR spectrum is more complicated (Figure S1a, Supporting Information). The thiophene protons would be expected to display two two-proton singlets reflecting the mirror plane perpendicular to the two rings. At ambient temperature, four broadened yet distinct one-proton singlets are observed at δ 9.68, 9.66, 9.64, and 9.63. Similarly, the four pyrrole protons would be expected to display two coupled, two-proton doublets reflecting the two sets of chemically different protons. At ambient temperature, four one-proton doublets are observed (J = 7.5 Hz for each) centered at δ 8.11, 8.02, 7.69, and 7.64. These data are consistent with two scenarios: (a) two isomeric molecules, one with a plane of symmetry through the thiophene rings and the other with a C_2 axis through the thiophene rings, or (b) a single molecule with two 4-sulfonatophenyl substituents, one 4-methyl-3-sulfonatophenyl and one 4-methyl-2-sulfonato substituent. The ^{13}C NMR spectrum of **15** displayed 20 distinct signals, one short of the 21 expected for the first scenario but far short of the 42 signals expected for the second.

When the ^{1}H NMR spectrum of **15** is acquired at 60 °C (Figure S1b, Supporting Information), the two sets of thiophene singlets collapse to two, two-proton singlets at δ 9.98 and 9.95, and the four pyrrole doublets collapse to an exchange-broadened singlet at δ 8.39 and a doublet at δ 7.98 with J = 7.5 Hz (Figure S1b, Supporting Information). At ambient temperature, the protons of the 4-sulfonatophenyl and 4-methylsulfonatophenyl groups are unresolved (Figure S1a, Supporting Information). At 60 °C, the 4-sulfonatophenyl protons resolve into an AA′BB′ pattern centered at δ 8.59 and 8.53 while the sulfonatotolyl groups resolve into a poorly resolved doublet at δ 9.08 with a coupling constant of ≈1 Hz, a broadened doublet at δ 8.93 with a coupling constant of 4.4 Hz (and presumably longer-range coupling to the proton at δ 9.08), and a well-resolved doublet at δ 8.88 with a coupling constant of 4.4 Hz. These results are only consistent with the first scenario involving two isomers with a symmetry element through the thiophene rings. If sulfonation of the tolyl groups was at the 2-position, hindered rotation of the 4-phenyl-2-sulfonatophenyl rings would create two isomers: one with both 2-sulfonato groups cis relative to the plane of the dithiaporphyrin ring (mirror plane of symmetry through the thiophenes) and one with the 2-sulfonato groups trans (C_2 axis through the thiophenes). In contrast, 3-sulfonatoaryl substituents would not experience the same hindered rotation. As a consequence, we have assigned the structure of **15** to have the 4-methyl-2-sulfonatophenyl substituents as shown in Chart 1. We have been unable to isolate or enrich one isomer relative to the other either through careful crystallization or through chromatographic separation.

Electronic Absorption Spectra of Core-Modified Porphyrins. The absorption spectra of 21,23-core-modified porphyrins **1**, **2**, **8**−**11**, and **13**−**17** in water are summarized in Table 1. A typical absorption spectrum is shown in Figure S2 (Supporting Information) for dithiaporphyrin **8**. The absorption maxima of band I, the long-wavelength band excited during PDT, are all at significantly longer wavelengths (689−717 nm) when compared to the band I maximum for either Photofrin or $TPPS_4$ (630 nm, ϵ = 3000 M^{-1} cm^{-1} for Photofrin and ϵ = 3900 M^{-1} cm^{-1} for $TPPS_4$) and have significantly stronger absorbances (Table 1). The spectral data for 21-thiaporphyrins **7** and **12** are also compiled in Table 1. The absorption maxima of band I for **7** (666 nm) and **12** (691 nm) are intermediate between the Photofrin and the 21,23-core-modified porphyrins.

Table 1. UV−Vis Band Maxima and Extinction Coefficients for $TPPS_4$, **1**, **2**, and **7**−**17** in Water, λ_{max} nm ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1})

compd	soret	band IV	band III	band II	band I
$TPPS_4$	411 (464)	513 (15.5)	549 (7.0)	577 6.5)	630 (3.9)
1	434 (190)	513 (19.3)	546 (5.5)	633 (2.0)	695 (4.0)
2	434 (221)	513 (22.4)	546 (6.2)	631 (2.2)	695 (4.5)
7	425 (445)	515 (22.4)	550 (7.2)	607 (4.0)	666 (4.2)
8	431 (476)	518 (15.7)	560 (8.3)	633 (2.3)	701 (4.6)
9	437 (443)	515 (34.9)	549 (10.8)	634 (2.6)	698 (7.1)
10	435 (243)	529 (20.4)	569 (10.5)	635 (3.6)	702 (6.3)
11	439 (65)	538 (10.3)	580 (5.1)	634 (3.0)	703 (3.8)
12	432 (224)	527 (12.6)	566 (5.4)	627 (2.5)	691 (3.2)
13	440 (270)	524 (19.7)	559 (7.0)	637 (2.1)	701 (4.2)
14	428 (79.7 447 (81.5)	515 (18.3)	577 (16.0)	635 (3.1)	716 (7.5)
15	433 (266)	515 (19.2)	549 (7.1)	627 (2.4)	689 (4.3)
16	434 (234)	514 (21.6)	549 (9.9)	634 (1.7)	699 (5.6)
17	442 (127)	533 (14.0)	573 (10.8)	645 (2.5)	717 (4.8)

The wavelength of the band I absorption maximum in the 21,23-core-modified porphyrins is little affected by the identity of the chalcogen atom. 21,23-Dithiaporphyrin (**1**) and 21,23-diselenaporphyrin (**2**) with four sulfonatophenyl substituents have identical band I maxima at 695 nm. Compounds **8**−**11** with 5,10-bis-4-fluorophenyl and 15,20-bis-4-sulfonatophenyl substituents and all combinations of sulfur and selenium atoms at the 21- and 23-positions have band I maxima that fall between 698 and 703 nm.

Quantum Yields for Fluorescence. In vivo fluorescence techniques have had a significant impact on the development of PDT with respect to the pharmacokinetics of sensitizers and with respect to PDT dosimetry.[19] Photofrin has been used in fluorescent diagnostics,[20] and the fluorescence of $TPPS_4$ has been extensively studied.[21] Several of the 21,23-core-modified porphyrins of this study were emissive although quantum yields for fluorescence (ϕ_F) were low (Table 2). A small (≤10 nm) Stokes shift was observed for the core-modified porphyrins.

The quantum yield standard used in these studies was a freshly prepared solution of $TPPS_4$ (ϕ_F = 0.12)[21a] dissolved in water. Compounds **1**, **8**, and **13** have values of ϕ_F of just under 0.01, and compound **17** has a value of ϕ_F of 0.003. A representative absorption/emission spectrum is shown for compound **8** in Figure S2 (Supporting Information). While these values of ϕ_F are low, they are sufficient to facilitate measurements of tissue distribution as described in the biological results below. The dimethylanilino analogue **14** was the only dithiaporphyrin examined that was nonemissive.

Table 2. Effective Concentrations To Give 50% Cell-Kill with 4 J cm^{-2} of Light (EC_{50}), Quantum Yields for the Generation of Singlet Oxygen [$\phi(^1O_2)$], Rates of Photobleaching (k_{bleach}), Flouresence Emission Maxima (λ_F), and Quantum Yields for Fluoresence (ϕ_F) for Sensitizers **1**, **2**, and **7**−**17** as Compared to Photofrin and $TPPS_4$

compd	E	E′	X	Ar[a]	EC_{50} (μM)	$\phi(^1O_2)$	$k_{bleach} \times 10^4\ s^{-1}$	λ_F (nm)	ϕ_F ($\pm\sigma$)
Photofrin					9.0		1.27 ± 0.01		
$TPPS_4$	NH	NH	SO_3Na	4-$C_6H_4SO_3Na$	125	0.71[b]			0.12[c]
1	S	S	SO_3Na	4-$C_6H_4SO_3Na$	30	0.50[d]	0.84 ± 0.02	700	8.6 (±0.4) × 10^{-3}
2	Se	Se	SO_3Na	4-$C_6H_4SO_3Na$	>100	0.17[d]		700	8.9 (± 0.4) × 10^{-4}
7	S	NH	SO_3Na	4-$C_6H_4SO_3Na$	>100	0.80 ± 0.02			
8	S	S	F	4-$C_6H_4SO_3Na$	1.6	0.74 ± 0.03	0.85 ± 0.03	703	6.9(±1.1) × 10^{-3}
9	S	Se	F	4-$C_6H_4SO_3Na$	2.1	0.65 ± 0.01			
10	Se	S	F	4-$C_6H_4SO_3Na$	1.2	0.64 ± 0.01		700	1.4 (±0.1) × 10^{-4}
11	Se	Se	F	4-$C_6H_4SO_3Na$	7.9	0.55 ± 0.02	1.44 ± 0.03		< 10^{-4}
12	S	NH	F	4-$C_6H_4SO_3Na$	2.1	0.78 ± 0.05	2.4 ± 0.2		
13	S	S	CF_3	4-$C_6H_4SO_3Na$	>100		0.13 ± 0.02	697	8.8 (±0.8) × 10-3
14	S	S	NMe_2	4-$C_6H_4SO_3Na$	0.64	0.60 ± 0.02	0.77 ±0.04		<10^{-4}
15	S	S	SO_3Na	$C_6H_4(CH_3)SO_3Na$[e]	12				
16	S	S	H	4-$C_6H_4OCH_2CO_2Na$	0.15		0.18 ± 0.02		
17	S	S	F	4-$C_6H_4OCH_2CO_2Na$	0.43	0.71 ± 0.02	0.16 ± 0.02	703	3.2(±0.1) × 10^{-3}

[a] 4-Substituted aryl. [b] Ref 23. [c] Ref 22a. [d] Ref 11. [e] 4-Methyl, 2-sulfonato.

The introduction of selenium into the porphyrin core greatly reduces the quantum yield for fluorescence due to heavy atom effects.[22] Diselenaporphyrin **2** is 10% as emissive in comparison to its dithiaporphyrin analogue **1**. Thiaselenaporphyrin **9** is 2% as emissive in comparison to its dithiaporphyrin analogue **8**. Diselenaporphyrin **11** gave no detectable emission.

Quantum Yields for Singlet Oxygen Generation. The generation of singlet oxygen appears to be important in the phototoxicity of the core-modified porphyrins.[11] The quantum yield for singlet oxygen generation [$\phi(^1O_2)$] by $TPPS_4$ has been reported to be 0.71,[23] while we have reported values of $\phi(^1O_2)$ for **1** and **2** to be 0.50 and 0.17, respectively (Table 2).[11] Values of $\phi(^1O_2)$ were measured for compounds **7**−**12**, **14**, and **17** and are compiled in Table 2. Values of $\phi(^1O_2)$ are uniformly higher for the 21,23-core-modified porphyrins with two 4-sulfonatonphenyl substituents relative to **1** and **2** with four 4-sulfonato groups. Values of $\phi(^1O_2)$ appear to be higher for the 21-thiaporphyrins relative to 21,23-core-modified porphyrins with identical substituents. Values of $\phi(^1O_2)$ for selenium-containing 21,23-core-modified porphyrins **9**−**11** are lower than for dithiaporphyrin **8**, which is somewhat surprising since spin−orbit effects from the heavy atom(s) would be more pronounced for **9**−**11** relative to **8**.[22] As we previously noted in comparing **1** and **2**,[11] one possible explanation is that the short, intramolecular Se···S contacts in **9** and **10** and the Se···Se contacts in **11** disrupt the planarity of the π-framework much more than the S···S contacts of the dithiaporphyrins. Such disruption may lead to less efficient intersystem crossing relative to the various photophysical routes to return to the ground state. Other substituent changes in the meso positions have little impact on values of $\phi(^1O_2)$ as represented by **14** [$\phi(^1O_2) = 0.60$] and **17** [$\phi(^1O_2) = 0.71$].

Photobleaching of 21,23-Core-Modified Porphyrins. One of the limitations of PDT dosimetry is the rate of bleaching of the photosensitizer.[1,2,10] Rates of photobleaching of core-modified porphyrins **1**, **8**, **11**−**14**, **16**, and **17** in phosphate-buffered saline (PBS) under conditions of constant initial absorbance between 570 and 750 nm using 570−750 nm light at 500 mW cm^{-2} are compiled in Table 2 and can be compared to that for Photofrin as shown in Figure S3 (Supporting Information) for several of these compounds. Photobleaching of 21-thiaporphyrin **12** occurs more rapidly than photobleaching of Photofrin while photobleaching of 21,23-diselenaporphyrin **11** occurs at a rate comparable to the photobleaching of Photofrin. 21,23-Dithiaporphyrins **1**, **8**, and **14** are less susceptible to photobleaching in comparison to Photofrin while dithiaporphyrins **13**, **16**, and **17** are nearly an order of magnitude more photostable in comparison to Photofrin.

Solubility Characteristics of Core-Modified Porphyrins. In comparing a series of compounds in order to establish structure−activity relationships, one must be concerned with the bioavailability of each of the individual compounds in the series. Of particular concern are the varying solubilities of different photosensitizer candidates, which can skew the comparison of results from studies in vitro and in vivo. The tetrasulfonato, disulfonato, and dicarboxylate derivatives of this study displayed varying degrees of aqueous solubility. The tetrasulfonato derivatives **1**, **2**, **7**, and **15** as well as disulfonato derivatives **8**−**13** were all soluble at 10 mg of sensitizer per milliliter of saline (≈10^{-2} M solutions), and the resulting solutions remained as such even upon storage at 5 °C. Dimethylanilino derivative **14** and dicarboxylate derivatives **16** and **17** were not soluble at 10 mg/mL in saline and gave stable solutions at 5 °C at concentrations of 1 mg of sensitizer per milliliter of saline (≈10^{-3} M).

Summary of Chemical and Photophysical Properties of Core-Modified Porphyrins. The 21- and 21,23-core-modified porphyrins **1**, **2**, and **7**−**17** of this study have chemical and photophysical properties that offer advantages as sensitizers for PDT. Synthetically, both the 21- and the 21,23-core-modified porphyrins can be prepared in pure form with a variety of aryl substituents and solubilizing groups as well as different combinations of heteroatoms at the 21- and 23-positions. The band I absorption maxima of all of these compounds are at longer wavelengths than the band I absorption maximum of either Photofrin or $TPPS_4$. The new core-modified porphyrins of this study (compounds **7**−**17**) produce singlet oxygen efficiently upon irradiation of band I. The meso substituents have little impact on values of $\phi(^1O_2)$. It should be noted that the 21,23-diselaporphyrins have lower values of $\phi(^1O_2)$ than other analogues perhaps reflecting the nonplanarity of the diselenaporphyrins. The 21,23-dithiaporphyrins exam-

ined for photobleaching in this study are all more photostable in comparison to Photofrin, and many of these species exhibit fluorescence quantum yields that are sufficient to assess cellular uptake and tissue distribution. Finally, all of the compounds **1**, **2**, and **7**−**17** are sufficiently water-soluble for the comparison of biological properties in vitro.

Biology

In Vitro Studies. Dark and Phototoxicities Against Colo-26 Cells in Culture. The core-modified porphyrins of this study and Photofrin were evaluated in culture for dark- and light-induced toxicities toward Colo-26 cells, a murine colon carcinoma cell line. Cell cultures were incubated for 24 h in the dark with various concentrations of sensitizer and were then washed prior to treatment with filtered 590−800 nm light for a total light dose of 4 J cm^{-2}. Light-treated cells and dark controls were incubated for 24 h, and cell survival was determined. Results are compiled in Table 2 as the effective concentration of sensitizer to give 50% cell kill with 4 J cm^{-2} of 590−800 nm light (EC_{50}). Values of EC_{50} were determined from a plot of the surviving fraction of cells vs concentration of sensitizer (Figures S4−S16 for **1**, **2**, and **7**−**17**, respectively, and Figure S17 for Photofrin, Supporting Information). None of the core-modified porphyrins displayed significant dark toxicity at concentrations ≤100 μM (Figures S4−S16, Supporting Information).

Against Colo-26 cells, all of the tetrasulfonated materials [dithiaporphyrin **1** (EC_{50} of 30 μM), diselenaporphyrin **2** (EC_{50} of >100 μM), thiaporphyrin **7** (EC_{50} of >100 μM), and dithiaporphyrin **15** (EC_{50} of 12 μM)] were less effective in comparison to Photofrin [EC_{50} of 5.7 mg/L, 9.0 μM (assuming a monomer)]. In analogy to the QSAR developed for porphyrin derivatives of $TPPS_4$,[12a,b,17] replacing the sulfonato groups on adjacent meso positions in the core-modified porphyrins should improve the relative phototoxicity. Replacing the 5- and 10-sulfonato substituents with fluoro substituents (compounds **8**−**12**) gave markedly lower values of EC_{50}. Diselenaporphyrin **11** with an EC_{50} value of 7.9 μM was comparable to Photofrin while compounds **8**−**10** and **12** with at least one sulfur heteroatom in the core were more effective than Photofrin with EC_{50} values of 1.2−2.1 μM. A comparison of Photofrin at several concentrations [2.5−10 μg/mL (4−16 μM)] with compound **8** at 3 μg/mL (3 μM) is shown in Figure 1.

Other substituent changes gave submicromolar values of EC_{50}. Replacing the sulfonato water-solubilizing groups of **8** with the carboxylate groups of **16** and **17** gave values of EC_{50} of 0.43 and 0.15 μM, respectively. Replacing the fluoro substituents of **8** with the dimethylamino groups of **14** gave a value of EC_{50} of 0.64 μM. In contrast, replacing the fluoro substituents of **8** with trifluoromethyl substituents in **13** gave greatly reduced phototoxicity with an EC_{50} of >100 μM.

Similar results were obtained with R3230AC rat mammary adenocarcinoma cells in vitro. Cell cultures were incubated for 24 h in the dark with 10 μM concentrations of Photofrin, **8**, **11**, **14**, or **17** and were then washed prior to treatment with filtered 570−800 nm light for a total light dose of 0.9 J cm^{-2}. Light-treated cells and dark controls were incubated for 24 h, and cell survival was determined. The surviving fraction 24 h postirradiation is shown graphically in Figure S18 (Supporting Information). As observed with Colo-26 cells (Table 2), **8**, **11**, **14**, and **17** were all more phototoxic than Photofrin against R3230AC cells. Furthermore, compounds **14** and **17** are more phototoxic than **8** and **11** toward R3230AC cells, which is also analogous to their behavior relative to **8** and **11** against Colo-26 cells (Table 2).

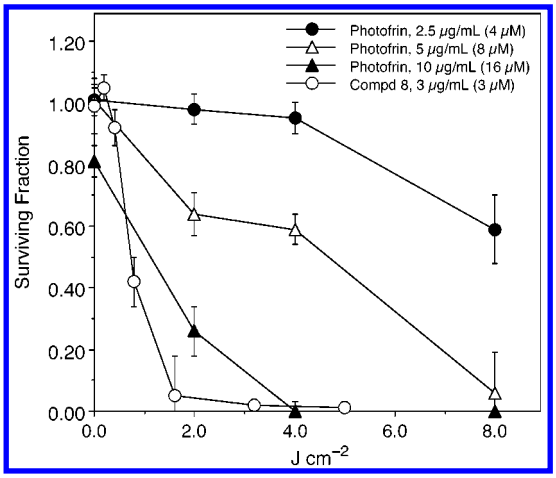


Figure 1. Comparison of dithiaporphyrin **8** and Photofrin photosensitization on the cell viability of cultured Colo-26 tumor cells. Data are expressed as the surviving fraction of viable cells for cells treated with various concentrations of Photofrin [2.5−10.0 μg/mL (4−16 μM)] and 630 nm light (75 mW cm^{-2}) or dithiaporphyrin **8** [3 μg/mL (3 μM)] and 694 nm light (75 mW cm^{-2}). Each data point represents the mean surviving fraction of viable cells calculated from at least three separate experiments performed in duplicate; bars are the SEM.

Although compounds **14**, **16**, and **17** were the most phototoxic materials against Colo-26 cells in vitro, these materials were not the most water-soluble in the series. For ease of formulation, initial in vivo studies were conducted with dithiaporphyrin **8** and diselenaporphyrin **11**, which readily dissolved in saline or 5% aqueous dextrose solutions.

In Vivo Studies. A. Initial Dark Toxicities. With porphyrin-related photosensitizers, the therapeutic dose for rodents is typically 1−5 mg/kg.[1,2] We chose a starting dose of 10 mg/kg to evaluate the core-modified porphyrins for dark toxicity. In groups of five BALB/c mice given a single intravenous injection of 10 mg (10 μmol)/kg of dithiaporphyrin **8** or 10 mg (10 μmol)/kg of diselenaporphyrin **11** as a saline solution, neither toxicity nor morbidity was observed. The animals were followed for 90 days postinjection under normal vivarium conditions (daily cycle of 12 h of fluorescent light/12 h of darkness). After the animals were sacrificed, no gross abnormalities were noted in the organs and tissues of sacrificed animals.

B. Distribution Studies with Dithiaporphyrin 8. Initial distribution studies were done at higher than therapeutic doses to facilitate the tracking of dithiaporphyrin **8** in various tissues. In BALB/c mice bearing Colo-26 tumors, the presence of **8** was measured by fluorescence spectroscopy at 1, 4, and 24 h postinjection of 5 mg (5 μmol)/kg of **8** (Figure 2). Fluorescence quenching in diselenaporphyrin **11** made similar studies impractical with this sensitizer. From these three time

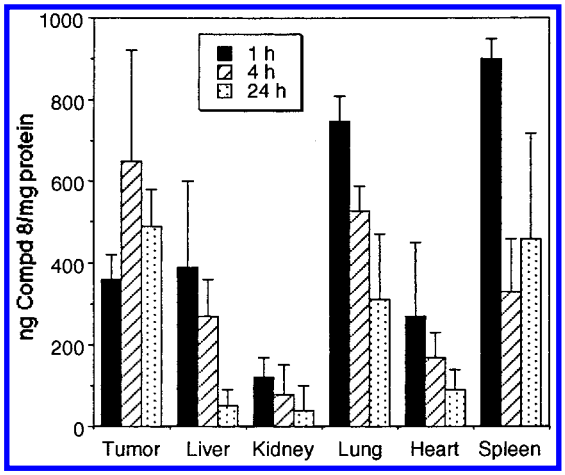


Figure 2. Tissue distribution of dithiaporphyrin **8** in BALB/c mice bearing Colo-26 tumors. Animals were given 5 mg (5 μmol)/kg of sensitizer and were sacrificed at the indicated time points (three animals per point). Tissues were cut, homogenized, and taken up in Solvable. Bars are the SEM.

points, the highest concentration of **8** in the tumor was found at 4 h, which suggested that the maximum concentration of **8** in tumors occurred between 1 and 24 h postinjection. In contrast, the concentration of **8** showed apparent decreases from the 1 h peak in the liver, kidney, lung, heart, and spleen. At 24 h, the concentration of **8** was higher in the tumor than in the liver, kidney, lung, and heart.

The uptake of dithiaporphyrin **8** in tumor was much greater than the uptake of dithiaporphyrin **1**. At a dose of 5 mg (5 μmol)/kg, a concentration of 19 $\pm$ 7 ng of **1**/g of protein was measured in tumors of BALB/c mice bearing Colo-26 tumors 24 h postinjection. In contrast, a concentration of 494 $\pm$ 90 ng of **8**/g of protein was measured in the tumors of animals 24 h postinjection of 5 mg (5 μmol) of **8**/kg (Figure 2), which represents a greater than 25-fold increase in photosensitizer uptake.

C. PDT with Dithiaporphyrin 8 and Diselenaporphyrin 11. On the basis of the EC_{50} values in Table 2, diselenaporphyrin **11** [EC_{50} of 7.9 μg/mL (7.9 μM)] should be comparable to Photofrin (EC_{50} of 9.0 μM) with respect to treatment dose while dithiaporphyrin **1** (EC_{50} > 100 μM) should require a significantly higher dose. In contrast, dithiaporphyrin **8** with an EC_{50} of 1.6 μM should require a significantly smaller treatment dose than either **11** or Photofrin.

BALB/c mice bearing Colo-26 tumors were given 10 mg (10 μmol)/kg of dithiaporphyrin **1**, 0.125 mg (0.13 μmol)/kg of dithiaporphyrin **8**, or 2.5 mg (2.5 μmol)/kg of diselenaporphyrin **11** as a 5% dextrose in water solution via tail vein injection. The animals were irradiated 4 h postinjection with 135 J cm^{-2} of 694 nm red light from a dye laser (75 mW cm^{-2} for 30 min). The time in days to 400 mm^3 tumor volume was noted for each animal in each group, and the results are presented as a Kaplan−Meyer plot in Figure 3. Of the five animals treated with dithiaporphyrin **8** and light, one cure was obtained, and the other four animals gave a mean time to 400 mm^3 of 18 $\pm$ 4 days, which is a 300% increase relative to untreated controls (6 $\pm$ 2 days, P < 0.001). Of the 10 animals treated with diselenaporphyrin **11** and light, three cures were obtained and the other seven animals gave a mean time to 400 mm^3 of 17 $\pm$ 3

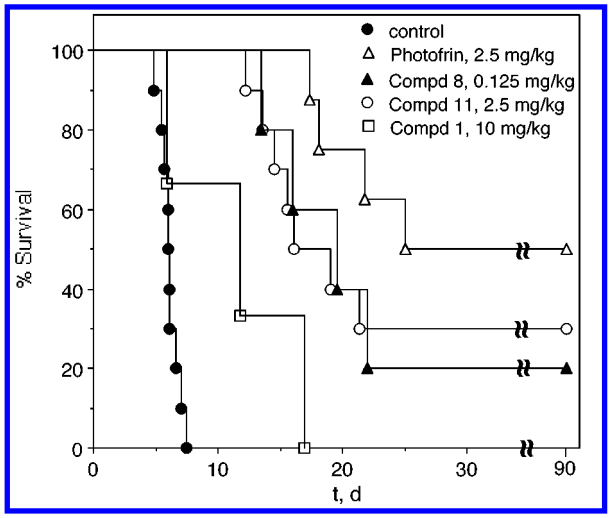


Figure 3. PDT with dithiaporphyrins **1** (open squares), **8** (filled triangles), and diselenaporphyrin **11** (open circles) against implanted Colo-26 tumors in BALB/c mice. Animals received 10 mg (10 μmol)/kg of **1**, 0.125 mg (0.13 μmol)/kg of **8**, or 2.5 mg (2.5 μmol)/kg of **11** and 135 J cm^{-2} of 694 nm light 4 h postinjection. Times were measured posttreatment until tumor volumes reached 400 mm^3. These data can be compared to a control group receiving neither drug nor light (filled circles) and to a group treated with 2.5 mg (4 μmol)/kg of Photofrin and 135 J cm^{-2} of 630 nm light (open triangles) 24 h postinjection.

days, which again is statistically different from untreated controls (P < 0.001). Of the four animals treated with dithiaporphyrin **1** and light, a mean time to 400 mm^3 of 13 $\pm$ 6 days was obtained, which represents a significant response relative to untreated controls (P < 0.014). However, no cures were obtained with **1** and the delay in tumor regrowth was significantly smaller than for **8** and **11**.

The results for PDT with **8** and **11** can be compared to those with Photofrin in the same animal model (Figure 3). BALB/c mice bearing Colo-26 tumors were given 2.5 mg (4.0 μmol)/kg of Photofrin as a 5% aqueous dextrose solution and were irradiated 24 h later with 135 J cm^{-2} of 630 nm laser light at a power of 75 mW cm^{-2}. The dose, interval between administration and irradiation, fluence, and fluence rate are published conditions for PDT with Photofrin.[2,8,9] Of the eight animals treated in this group, four cures were obtained and the other four animals gave a mean time to 400 mm^3 of 21 $\pm$ 4 days, which is not significantly different than the results with either **8** at a dose of 0.125 mg/kg or **11** at a dose of 2.5 mg/kg and irradiation 4 h after sensitizer injection (P > 0.59).

It should be noted that no aspect of PDT with either **8** or **11** has yet been studied to achieve optimization (i.e., the sensitizer dose, the delivery vehicle, the interval between sensitizer administration and irradiation, light dose, fluence, and application of fractionated irradiation). Despite this, PDT with either **8** or **11** clearly gives efficacy comparable to that of Photofrin using published conditions.[2,8,9]

D. Clearance Studies Using Ear-Swelling Response as an End Point. With Photofrin and related porphyrin sensitizers, long-term acute cutaneous photosensitivity is a significant side effect in their clinical use.[9] The time course of acute cutaneous photosensitivity following administration of 0.125 mg (0.13 μmol)/kg

of **8** or 5.0 mg (5 μmol)/kg of **11** was examined using the established murine ear-swelling response (ESR).[24] The data for **8** and **11** can be compared to the ESR observed in Photofrin-treated [5 mg (8 μmol)/kg] mice as reported in ref 24. The absolute ESR at day 1 for **8** at 0.125 mg (0.13 μmol)/kg or for **11** at 5.0 mg (5 μmol)/kg was on the order of 0.11−0.13 mm of swelling above the normal untreated ear thickness of 0.25−0.30 mm. The absolute swelling observed with Photofrin-treated animals on day 1 was on the order of 0.20−0.25 mm.[24] The ESR disappeared between days 3 and 4 with **8** at 0.125 mg/kg and between days 5 and 7 with **11** at 5.0 mg/kg. Even at 31 days, there remained a positive ESR in Photofrin-treated animals.[24] Importantly, concentrations of **8** or **11** that gave comparable results to Photofrin for PDT efficacy gave significantly reduced duration of cutaneous photosensitivity.

Summary of Biological Properties. The sulfonated and carboxylated core-modified porphyrins examined in this study are all sufficiently soluble at the concentrations examined for the PDT studies in vitro to allow a comparison of structure and activity. A comparison of sulfonates **1**, **8**, and **11** with Photofrin demonstrated that relative phototoxicity in vitro (against Colo-26 and R3230AC cells) roughly translates to relative effectiveness for PDT in vivo. It should be possible to optimize sensitizer performance through structure−activity studies in vitro in the sulfonato series. The dicarboxylate derivatives **16** and **17** were even more effective than sulfonato derivatives **1**, **2**, and **7**−**15** as PDT sensitizers in vitro although the dicarboxylates were less water-soluble. We are evaluating delivery vehicles for dicarboxylates **16** and **17** (as well as other carboxylate derivatives) and for dimethylanilino derivative **14** in order to evaluate their effectiveness as PDT sensitizers in vivo.

If one sulfur atom is in the core, the second heteroatom at the 21- or 23-position can be selected from NH, S, or Se with little effect on phototoxicity in vitro. However, one structural feature that appears to be important for increased phototoxicity in vitro in the sulfonato series is the presence of hydrogen bond-accepting groups on two of the aromatic rings. The fluorophenyl substituents of **8**−**12** and the dimethylanilino substituents of **14** contribute to lower values of EC_{50} relative to the (trifluoromethyl)phenyl substituents of **13** or the tolyl substituents of **15**.

Studies in vivo with dithiaporphyrin **8** and diselenaporphyrin **11** suggest that effective core-modified porphyrin sensitizers can be developed that (i) can be prepared with well-defined structure and purity; (ii) are amenable to structure−activity studies to identify better materials; (iii) absorb light of longer wavelengths than Photofrin, which increases the effective depth of penetration of light; (iv) have minimal dark toxicity; (v) localize in the tumor; and (vi) have limited cutaneous photosensitivity, which limits PDT with Photofrin. We are currently optimizing PDT with our lead candidates **8**, **11**, **14**, **16**, and **17** as well as examining new derivatives.

Experimental Section

General Methods. Solvents and reagents were used as received from Sigma-Aldrich Chemical Co. (St. Louis, MO) unless otherwise noted. Cell culture medium was purchased from GIBCO (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Atlanta, GA). Concentration in vacuo was performed on a Büchi rotary evaporator. NMR spectra were recorded at 30.0 °C on a Varian Gemini-300, Inova 400, or Inova 500 instrument with residual solvent signal as the internal standard: $CDCl_3$ (δ 7.26 for proton, δ 77.0 for carbon). Infrared spectra were recorded on a Perkin-Elmer FT-IR instrument. UV−visible near-IR spectra were recorded on a Perkin-Elmer Lambda 12 spectrophotometer or on a Sequential DX17 MV Stopped-Flow Spectrometer (Applied Photophysics, Leatherhead, U.K.). Both were equipped with a circulating constant-temperature bath for the sample chambers. Elemental analyses were conducted by Atlantic Microanalytical, Inc. Electrospray and MALDI mass spectrometry were conducted by the Campus Chemical Instrumentation Center of The Ohio State University (Columbus, OH). Compounds **1**−**3a**,**b** were prepared as described in ref 11.

General Procedure for the Preparation of 2,5-Bis(arylhydroxymethyl)thiophenes and Selenophenes. Preparation of 2,5-Bis[(4-fluorophenyl)hydroxymethyl]thiophene (3c). Thiophene (8.4 g, 0.10 mol) was added to a solution of *n*-butyllithium (177 mL, 1.6 M in hexanes, 0.30 mol) and TMEDA (45 mL, 0.31 mol) in 500 mL of hexanes, under an argon atmosphere. The reaction mixture was heated at reflux for 2 h, cooled to ambient temperature, and transferred via a cannula to a pressure-equalizing addition funnel. This dilithiothiophene suspension was then added dropwise to a 0 °C solution of 4-fluorobenzaldehyde (37.2 g, 0.30 mol) in THF (500 mL), which had been dried over basic alumina and degassed with argon for 15 min. After the addition was complete, the mixture was allowed to warm to room temperature, 750 mL of a 1 M solution of ammonium chloride was added, and the organic phase was separated. The aqueous phase was extracted with ether (3 × 400 mL). The combined organic extracts were washed with water (3 × 500 mL) and brine (500 mL), dried over $MgSO_4$, and concentrated to a yellow oil. The crude product was recrystallized from toluene to give 14.3 g (43%) of white crystalline product of one diasteromer (meso or d,l); mp 77−79 °C (literature mp[14a] 103−104 °C). IR (KBr): 3402 br, 1663, 1516, 1225, 1015 cm^{-1}. FAB(+) MS: *m*/*z* 331 ($C_{18}H_{14}F_2O_2S$ + H, M + 1). Anal. C, H.

Preparation of 2,5-Bis[(4-fluorophenyl)hydroxymethyl]selenophene (3d). Selenophene (7.5 g, 0.06 mol) was treated with *n*-butyllithium (89 mL, 1.6 M in hexanes, 0.15 mol) and TMEDA (23 mL, 0.15 mol) in 250 mL of hexanes as described. The dilithioselenophene suspension was then slowly added dropwise to a solution of 4-fluorobenzaldehyde (37.2 g, 0.3 mol) in THF (300 mL) as described. Following workup, the crude product was recrystallized from toluene to give 8.42 g (37%) of **3d** as a white crystalline product of one diasteromer (meso or d,l); mp 95−95.5 °C. IR (KBr): 3246, 1675, 1613, 1512, 1225 cm^{-1}. FAB(+) MS: *m*/*z* 381 ($C_{18}H_{14}F_2O_2{}^{80}Se$ + H, M + 1). Anal. C, H.

Preparation of 2,5-Bis[(4-trifluoromethylphenyl)hydroxymethyl]thiophene (3e). Thiophene (1.68 g, 0.020 mol) was treated with *n*-butyllithium (31 mL, 1.6 M in hexanes, 0.050 mol) and TMEDA (10 mL, 0.07 mol) in 100 mL of hexanes as described. The dilithiothiophene suspension was added dropwise to a solution of 4-trifluorotolualdehyde (7.66 g, 0.044 mol) in THF (125 mL) as described. Following workup, the crude product was recrystallized from toluene to give 4.17 g (52%) of **3e** as a white crystalline solid; mp 160−162 °C. IR (KBr): 3377 (br), 1338, 1118, 1072 cm^{-1}. High-resolution Q-TOF MS: *m*/*z* 455.0524 (calcd for $C_{20}H_{14}F_6O_2S$ + Na, 455.0516). Anal. C, H.

Preparation of 2,5-Bis[(4-*N*,*N*-dimethylaminophenyl)-hydroxymethyl]thiophene (3f). Thiophene (4.2 g, 0.050 mol) was treated with *n*-butyllithium (78 mL, 1.6 M in hexanes, 0.13 mol) and TMEDA (19 mL, 0.13 mol) in 200 mL of hexanes as described. The dilithiothiophene suspension was added dropwise to a solution of 4-dimethylaminobenzaldehyde (19.4 g, 0.13 mol) in THF (250 mL) as described. Following workup, the crude product was recrystallized from toluene to give 12.34

g (65%) of **3f** as a white crystalline solid; mp 101−103 °C. FAB(+) MS: *m/z* 383 ($C_{22}H_{26}N_2O_2S$ + H, M + 1). Anal. C, H, N.

Preparation of 2,5-Bis[(4-tolyl)hydroxymethyl]thiophene (3g). Thiophene (4.2 g, 0.050 mol) was treated with *n*-butyllithium (78 mL, 1.6 M in hexanes, 0.13 mol) and TMEDA (19 mL, 0.13 mol) in 200 mL of hexanes as described. The dilithiothiophene suspension was added dropwise to a solution of 4-methylbenzaldehyde (15.6 g, 0.13 mol) in THF (250 mL) as described. Following workup, the crude product was recrystallized from toluene to give 13.2 g (82%) of **3g** as a white crystalline product; mp 92−94 °C. IR (KBr): 3283 (br), 2920, 2877, 1451, 1009 cm^{-1}. High-resolution Q-TOF MS: *m/z* 347.1079 (calcd for $C_{20}H_{20}O_2S$ + Na, 347.1082). Anal. C, H.

Preparation of 2,5-Bis[(4-methoxyphenyl)hydroxymethyl]thiophene (3h). Thiophene (5.12 g, 0.060 mol) was treated with *n*-butyllithium (78 mL, 1.6 M in hexanes, 0.13 mol) and TMEDA (19 mL, 0.13 mol) in 200 mL of hexanes as described. The dilithiothiophene suspension was added dropwise to a solution of 4-methoxybenzaldehyde (17.0 g, 0.125 mol) in THF (250 mL) as described. Following workup, the crude product was recrystallized from toluene to give 8.75 g (41%) of **3h** as a white crystalline product; mp 125−127 °C (literature mp[14a] 154 °C). FAB(+) MS: *m/z* 357 ($C_{20}H_{20}O_4S$ + 1, M + 1). Anal. C, H.

General Procedure for the Preparation of 2,5-Bis(1-aryl-1-pyrrolomethyl)chalcogenophenes. Preparation of 2,5-Bis(1-phenyl-1-pyrrolomethyl)thiophene (4a). Compound **3a**[11] (1.3 g, 4.0 mmol) was dissolved in excess pyrrole (10.6 mL), and argon was bubbled through it. Boron trifluoride etherate was added (0.1 mL), and the resulting mixture was allowed to stir for 1 h. The reaction was stopped by the addition of CH_2Cl_2 (100 mL) followed by 40% NaOH (25 mL). The organic layer was separated, washed with water (3 × 100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated. The excess pyrrole was removed via vacuum distilled at ambient temperature. The residual oil was purified via chromatography on silica gel eluted with 75:25 hexanes/ethyl acetate. The yellow band was collected to give a yellow oil, which was recrystallized from ethyl acetate/hexanes to give 0.74 g (46%) of **4a** as a white solid; mp 98−100 °C. IR (KBr): 3510, 2978, 1716, 1521, 1363, 1222 cm^{-1}. FAB(+) MS: *m/z* 395 ($C_{26}H_{22}N_2S$ + H, M^+ + 1). Anal. C, H, N.

Preparation of 2,5-Bis(1-phenyl-1-pyrrolomethyl)selenophene (4b). 2,5-Bis-(phenylhydroxymethyl)selenophene[11] (1.21 g, 3.5 mmol) and pyrrole (10.8 mL) were treated with boron trifluoride etherate (0.1 mL) as described. The residual oil was purified via chromatography on silica gel eluted with 75:25 hexanes/ethyl acetate and was recrystallized from ethyl acetate/hexanes to give 0.70 g (51%) of **4b** as a white solid; mp 109−111 °C. IR (KBr): 3426, 1700, 1628, 1457 cm^{-1}. FAB(+) MS: *m/z* 443 ($C_{26}H_{22}N_2{}^{80}Se$ + H, M + 1). Anal. C, H, N.

Preparation of 2,5-Bis[1-(4-fluorophenyl-1-pyrrolomethyl]thiophene (4c). Compound **3c**[14a] (0.40 g, 1.5 mmol) and pyrrole (4 mL) were treated with boron trifluoride etherate (0.080 mL) as described. The residual oil was purified via chromatography on silica gel eluted with 80:20 hexanes/ethyl acetate to give 0.40 g (78%) of **4c** as a colorless oil that was used without further purification. HRMS: *m/z* 430.1305 (calcd for $C_{26}H_{20}F_2N_2S$, 430.1315). Anal. C, H, N.

Preparation of 2,5-Bis[1-(4-methoxyphenyl-1-pyrrolomethyl]thiophene (4d). Compound **3h**[14a](0.41 g, 1.15 mmol) and pyrrole (4 mL) were treated with boron trifluoride etherate (0.080 mL) as described. The residual oil was purified via chromatography on silica gel eluted with 80:20 hexanes/ethyl acetate to give 0.48 g (92%) of **4d** as a colorless oil that was used without further purification. HRMS: *m/z* 454.1694 (calcd for $C_{28}H_{26}N_2O_2S$, 454.1715). Anal. C, H, N.

General Procedure for the Preparation of 5,10,15,20-Tetra(aryl)-21,23-core-modified Porphyrins. Preparation of 5,10-Bis-4-fluorophenyl-15,20-diphenyl-21,23-dithiaporphyrin (5a). Compounds **4a** (1.33 g, 3.6 mmol) and **3c** (1.12 g, 3.6 mmol) were dissolved in 1 L of CH_2Cl_2 that had been degassed under a stream of Ar bubbles for 20 min. TCBQ (4.52 g) was added, and the reaction vessel was covered with aluminum foil, followed by purging of the system with argon. Boron trifluoride etherate (0.2 mL) was added, and the resulting mixture was heated at reflux for 1 h. The reaction mixture was cooled and concentrated, and the residue was redissolved in minimal CH_2Cl_2. The resulting solution was purified via chromatography on basic alumina eluted with CH_2Cl_2. The first red band was collected, and the product was washed with acetone. The crude product was then recrystallized from CH_2Cl_2/MeOH to give 0.252 g (11%) of **5a** as a purple solid, which was recrystallized from toluene to give purple needles; mp >300 °C. IR (KBr): 3446, 1679, 1628, 1472 cm^{-1}. FAB(+) MS: *m/z* 685 ($C_{44}H_{26}F_2N_2S_2$ + H, M + 1). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-diphenyl-21,23-diselenaporphyrin (5b). Compounds **4b** (1.48 g, 3.4 mmol) and **3d** (1.27 g, 3.4 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (4.52 g) and then boron trifluoride etherate (0.2 mL) as described. The crude product was recrystallized from CH_2Cl_2/MeOH to give 0.257 g (12%) of **5b** as a purple solid; mp >300 °C. FAB(+) MS: *m/z* 781 ($C_{44}H_{26}F_2N_2{}^{80}Se_2$ + H, M + 1). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-diphenyl-21-thia-23-selenaporphyrin (5c). Compounds **4b** (1.59 g, 3.6 mmol) and **3c** (1.20 g, 3.6 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (4.5 g) and then boron trifluoride etherate (0.2 mL) as described. The crude product was recrystallized from CH_2Cl_2/MeOH to give 0.42 g (16%) of **5c** as a purple solid; mp >300 °C. High-resolution Q-TOF MS: *m/z* 733.1034 (calcd for $C_{44}H_{26}F_2N_2S{}^{80}Se$ + H, 733.1027). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-diphenyl-21-selena-23-thiaporphyrin (5d). Compounds **4a** (1.69 g, 4.3 mmol) and **3d** (1.62 g, 4.3 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (4.41 g) and then boron trifluoride etherate (0.2 mL) as described. The crude product was recrystallized from CH_2Cl_2/MeOH to give 0.19 g (6%) of **5d** as a purple solid; mp >300 °C. High-resolution Q-TOF MS: *m/z* 733.1034 (calcd for $C_{44}H_{26}F_2N_2S{}^{80}Se$ + H, 733.1027). Anal. C, H, N.

Preparation of 5,10-Bis-4-(trifluoromethyl)phenyl-15,-20-diphenyl-21,23-dithiaporphyrin (5e). Compounds **4a** (1.70 g, 4.3 mmol) and **3e** (1.73 g, 4.3 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (4.5 g) and boron trifluoride etherate (0.2 mL) as described. The crude product was recrystallized from MeOH/CH_2Cl_2 to give 0.075 g (2%) of **5e**, which was recrystallized from toluene to give purple needles; mp >300 °C. FAB(+) MS: *m/z* 785 ($C_{46}H_{26}F_6N_2S_2$ + H, M + 1). Anal. C, H, N.

Preparation of 5,10-Bis-4-(*N*,*N*-dimethylamino)phenyl-15,20-diphenyl-21,23-dithiaporphyrin (5f). Compounds **4a** (0.71 g, 1.8 mmol) and **3f** (0.69 g, 1.8 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (2.20 g) and boron trifluoride etherate (0.1 mL) as described. The crude product was purified via chromatography on basic alumina eluted with CH_2Cl_2. The crude product was recrystallized from CH_2Cl_2/MeOH to give 0.225 g (17%) of **5f** as a purple solid; mp >300 °C. High-resolution Q-TOF MS: *m/z* 735.2645 (calcd for $C_{48}H_{38}N_4S_2$ + H, 735.2616). Anal. C, H, N.

Preparation of 5,10-Bis-4-tolyl-15,20-diphenyl-21,23-dithiaporphyrin (5g). Compounds **4a** (1.42 g, 3.6 mmol) and **3g** (1.17 g, 3.6 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (4.52 g) and boron trifluoride etherate (0.2 mL) as described. The crude product was recrystallized from CH_2Cl_2/MeOH to give 0.52 g (22%) of 5 g as a purple solid; mp >300 °C. High-resolution Q-TOF MS: *m/z* 677.2094 (calcd for $C_{46}H_{32}N_2S_2$ + H, 677.2085). Anal. C, H, N.

Preparation of 5,10-Bis-4-methoxyphenyl-15,20-diphenyl-21,23-dithiaporphyrin (5h). Compounds **4a** (1.42 g, 3.6 mmol) and **3h** (1.28 g, 3.6 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (4.40 g) and boron trifluoride etherate (0.2 mL) as described. The crude product was recrystallized from CH_2Cl_2/MeOH to give 0.71 g (29%) of **5h** as a purple solid; mp >300 °C. High-resolution Q-TOF MS: *m/z* 677.2094 (calcd for $C_{46}H_{32}N_2S_2$ + H, 677.2085). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-bis-4-methoxyphenyl-21,23-dithiaporphyrin (5l). Compounds **4c** (1.00 g, 2.33 mmol) and **3i** (0.83 g, 2.33 mmol) in 750 mL of CH_2Cl_2 were treated with TCBQ (2.2 g, 8.95 mmol) and boron trifluoride etherate (0.15 mL, 1.2 mmol) as described. The crude product was recrystallized from MeOH/CH_2Cl_2 to give 0.50 g (29%) of **5l** as a purple solid; mp >300 °C. High-resolution Q-TOF MS: *m*/*z* 745.1793 (calcd for $C_{46}H_{30}F_2N_2O_2S_2$ + H, 745.1795). Anal. C, H, N.

Preparation of 5,10,15,20-Tetraphenyl-21-thiaporphyrin (6a).[18] Pyrrole (1 mL, 0.015 mol), benzaldehyde (1 mL, 0.01 mol), and **3a** (1.48 g, 5.00 mmol) in 1 L of CH_2Cl_2 were treated as described to give 0.38 g (12%) of **6a** as dark purple crystals; mp > 300 °C (literature mp[18] >300 °C). IR (KBr): 3010, 2977, 1717, 1512, 1370, 1225 cm^{-1}. FAB(+) MS: *m*/*z* 632 ($C_{44}H_{29}N_3S$ + H, M + 1).

Preparation of 5,10-Bis-4-fluorophenyl-15,20-diphenyl-21-thiaporphyrin (6b). Pyrrole (1 mL, 0.015 mol), benzaldehyde (1 mL, 0.01 mol), and **3c** (1.66 g, 5.0 mmol) in 1 L of CH_2Cl_2 were treated with TCBQ (4.52 g) and boron trifluoride etherate (0.2 mL) as described. The crude product was recrystallized from CH_2Cl_2/hexanes to give 0.25 g (8%) of **6b** as a purple solid; mp >300 °C. FAB(+) MS: *m*/*z* 668 ($C_{44}H_{27}F_2N_3S$ + H, M + 1). Anal. C, H, N.

Preparation of 5,10-Diphenyl-15,20-bis-4-hydroxyphenyl-21,23-dithiaporphyrin (5i). A solution of porphyrin **5h** (312 mg, 0.44 mmol) in 100 mL of 1,2-dichloroethane was degassed with a stream of argon. Boron tribromide methyl sulfide complex (1 M in CH_2Cl_2, 8 mL, 8 mmol) was added, and the resulting solution was heated at reflux for 15 h. The reaction mixture was cooled to ambient temperature, and 50 mL of MeOH was added. After 15 min, the reaction mixture was concentrated, and 200 mL of brine and 200 mL of ethyl acetate were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude solid was washed with several portions of MeOH to give 170 mg (57%) of the phenolic porphyrin **5i**, which was recrystallized from CH_2Cl_2/toluene to give purple needles; mp >300 °C. High-resolution Q-TOF MS: *m*/*z* 717.1475 (calcd for $C_{44}H_{28}N_2O_2S_2$ + H, 717.1482). Anal. C, H, N.

Preparation of 5,10-Diphenyl-15,20-bis-(4-ethyl phenoxyacetate)-21,23-dithiaporphyrin (5j). Porphyrin **5i** (126 mg, 0.185 mmol), 1.5 g of K_2CO_3, and 2 mL of ethyl bromoacetate in 50 mL of acetone were heated at reflux for 15 h until **5i** was consumed. The reaction mixture was cooled to ambient temperature, and the K_2CO_3 was removed by filtration. The filter cake was washed with THF until the filtrate was colorless. The combined filtrates were concentrated. The crude product was washed with MeOH to give 79 mg (50%) of **5j** as a purple solid; mp 255−257 °C. High-resolution Q-TOF MS: *m*/*z* 751.2084 (calcd for $C_{52}H_{40}N_2O_6S_2$ + H, 751.2089). Anal. C, H, N.

Preparation of 5,10-Diphenyl-15,20-bis-(4-phenoxyacetic acid)-21,23-dithiaporphyrin (5k). Porphyrin **5j** (75 mg, 0.088 mmol) was dissolved in 30 mL of THF and 285 mg (0.14 mmol) of NaOH in 13 mL of H_2O was added. The resulting solution was stirred at ambient temperature for 15 h. After the solution was acidified with 3 mL of acetic acid, 50 mL of H_2O and 100 mL of ethyl acetate were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was washed with several portions of Et_2O to give 63.5 mg (91%) of **5k**, which was recrystallized from EtOAc to give purple needles; mp >300 °C. High-resolution Q-TOF MS: *m*/*z* 797.1778 (calcd for $C_{48}H_{32}N_2O_6S_2$ + H, 797.1780). Anal. C, H, N.

Preparation of 5,10-Diphenyl-15,20-bis-(4-phenoxyacetic acid)-21,23-dithiaporphyrin Disodium Salt (16). Porphyrin **5k** (55.3 mg, 0.0694 mmol) was dissolved in 30 mL of THF, and 1.46 mL (0.146 mmol) of 0.10 M aqueous NaOH was added. The solution was stirred for 15 h at ambient temperature. The purple precipitate was collected by filtration, washed with several portions of THF, and dried to give 41 mg (70%) of porphyrin **16**; mp >300 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 9.72 (s, 2H), 9.60 (s, 2H), 8.61 (d, 2H, J = 4.5 Hz), 8.53 (d, 2H, J = 4.5 Hz), 8.10−8.17 (m, 4H), 8.06 (d, 4H, J = 8.1 Hz), 7.74−7.80 (m, 6H), 7.37 (d, 4H, J = 8.1 Hz), 4.62 (s, 4H). ^{13}C NMR (75 MHz, CD_3OD): δ 176.51, 160.72, 158.13, 157.76, 149.46, 148.81, 142.39, 136.87, 136.46, 135.82, 135.64, 135.19, 135.10, 134.56, 129.37, 128.69, 115.13, 68.78. High-resolution Q-TOF MS: *m*/*z* 841.1427 (calcd for $C_{48}H_{30}N_2Na_2O_6S_2$ + H, 841.1419). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-bis-4-hydroxyphenyl-21,23-dithiaporphyrin (5m). A solution of porphyrin **5l** (312 mg, 0.44 mmol) in 100 mL of 1,2-dichloroethane was degassed with a stream of argon. Boron tribromide methyl sulfide complex (1 M in CH_2Cl_2, 8 mL, 8 mmol) was added, and the resulting solution was heated at reflux for 15 h. The reaction mixture was cooled to ambient temperature, and 50 mL of MeOH was added. After 15 min, the reaction mixture was concentrated, and 200 mL of brine and 200 mL of ethyl acetate were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude solid was washed with several portions of MeOH to give 170 mg (57%) of the phenolic porphyrin **5m**, which was recrystallized from CH_2Cl_2/toluene to give purple needles; mp >300 °C. IR (KBr): 3400 (br), 1603, 1508, 1223, 1157 cm^{-1}. High-resolution Q-TOF MS: *m*/*z* 717.1475 (calcd for $C_{44}H_{26}F_2N_2O_2S_2$ + H, 717.1482). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-bis(4-ethyl phenoxyacetate)-21,23-dithiaporphyrin (5n). Porphyrin **5m** (185 mg, 0.26 mmol), 1.5 of K_2CO_3, and 2 mL of ethyl bromoacetate in 50 mL of acetone were heated at reflux for 15 h until **5m** was consumed. The reaction mixture was cooled to ambient temperature, and the K_2CO_3 was removed by filtration. The filter cake was washed with THF until the filtrate was colorless. The combined filtrates were concentrated. The crude product was washed with MeOH to give 175 mg (76%) of **5n** as a purple solid; mp 255−257 °C. IR (KBr): 1757, 1603, 1511, 1223, 1195, 1180 cm^{-1}. High-resolution Q-TOF MS: *m*/*z* 889.2198 (calcd for $C_{52}H_{38}F_2N_2O_6S_2$ + H, 889.2217). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-bis(4-phenoxyacetic acid)-21,23-dithiaporphyrin (5o). Porphyrin **5n** (190 mg, 0.21 mmol) was dissolved in 30 mL of THF, and 200 mg (2.5 mmol) of NaOH in 10 mL of H_2O was added. The resulting solution was stirred at ambient temperature for 15 h. After the solution was acidified with 1 mL of acetic acid, 100 mL of H_2O and 100 mL of ethyl acetate were added. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was washed with several portions of Et_2O to give 164 mg (93%) of **5o** as a purple solid; mp >300 °C. IR (KBr): 3420 (br), 1730, 1602, 1512, 1223 cm^{-1}. High-resolution Q-TOF MS: *m*/*z* 833.1589 (calcd for $C_{48}H_{30}F_2N_2O_6S_2$ + H, 883.1591). Anal. C, H, N.

Preparation of 5,10-Bis-4-fluorophenyl-15,20-bis(4-phenoxyacetic acid)-21,23-dithiaporphyrin Disodium Salt (17). Porphyrin **5o** (140 mg, 0.17 mmol) was dissolved in 30 mL of THF, and 1.4 mL (0.34 mmol) of 0.24 M aqueous NaOH was added. The solution was stirred for 15 h at ambient temperature. The purple precipitate was collected by filtration, washed with several portions of THF, and dried to give 140 mg (91%) of porphyrin **17**; mp >300 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 9.79 (s, 2H), 9.63 (s, 2H), 8.68 (d, 2H, J = 4.5 Hz), 8.58 (d, 2H, J = 4.5 Hz), 8.07−8.24 (m, 8H), 7.56 (t, 4H, J = 8.4 Hz), 7.44 (d, 4H, J = 8.7 Hz), 4.70 (s, 4H). ^{13}C NMR (75 MHz, CD_3OD): δ 176.51, 164.61 (d, J = 245 Hz), 160.74, 158.18, 157.76, 149.59, 148.83, 138.46 (d, J = 3 Hz), 136.98, 136.78 (d, J = 8 Hz), 136.49, 136.33, 135.99, 135.83, 134.92, 134.49, 133.82, 115.70, 115.28 (d, J = 20 Hz), 68.78. High-resolution Q-TOF MS: *m*/*z* 877.1262 (calcd for $C_{48}H_{28}F_2N_2Na_2O_6S_2$ + H, 877.1230). Anal. C, H, N.

General Procedure for the Sulfonation of Core-Modified Porphyrins. Preparation of 5,10-Bis-4-sulfonatophenyl-15,20-bis-4-fluorophenyl-21,23-dithiaporphyrin Disodium Salt (8). Compound **5a** (0.53 g, 0.77 mmol) was dissolved in excess H_2SO_4 (26 mL) and allowed to stir at 100 °C overnight. The acid was slowly neutralized with concentrated NaOH until the solution was slightly basic. An equal volume of MeOH was added, and the solid Na_2SO_4 was removed by filtration. The filtrate was concentrated, and the residue was dissolved in acetone. The resulting solution was chilled precipitating more Na_2SO_4, which was removed via filtration. The acetone solution was concentrated, and the residue was dissolved in a minimal amount of water. The resulting solution was subjected to reverse phase chromatography eluting with MeOH. The purple band was collected, and the residue was recrystallized from 10% aqueous MeOH to give 0.34 g (49%) of **8** as a metallic purple solid; mp >300 °C. ^{1}H NMR (CD_3OD, 500 MHz): δ 9.71 (s, 2H), 9.67 (s, 2H), 8.62 (s, 4H), 8.32 (AA′, 4H, J = 8 Hz), 8.26 (BB′, 4H, J = 8 Hz), 8.18 (m, 4H), 7.57 (t, 4H, J = 9 Hz). ^{13}C NMR (CD_3OD, 125 MHz): δ 164.63 (d, J = 247 Hz), 157.81, 157.61, 149.24, 148.96, 146.46, 143.99, 138.23, 136.85 (d, J = 8 Hz), 136.84, 136.68, 135.53, 135.43, 135.05, 134.50, 126.33, 115.60 (d, J = 22 Hz). IR (KBr): 3453, 1696, 1653, 1482, 1358, 1184 cm^{-1}. MALDI TOF MS: m/z 888 ($C_{44}H_{24}F_2N_2Na_2O_6S_4$ + H, M + 1), 866 ($C_{44}H_{25}F_2N_2NaO_6S_4$ + H), 844 ($C_{44}H_{26}F_2N_2O_6S_4$ + H). Anal. C, H, N.

Preparation of Tetra-4-sulfonatophenyl-21-thiaporphyrin Tetrasodium Salt (7). Compound **6a** (0.20 g, 0.30 mmol) was treated as described to give 0.27 g (84%) of **7** as dark purple crystals; mp > 300 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 9.97 (s, 2H), 9.02 (s, 2H), 8.69 (d, 2H, J = 5 Hz), 8.61 (d, 2H, J = 5 Hz), 8.34 (AA′, 8H), 8.28 (BB′, 8H). ^{13}C NMR (CD_3OD, 75 MHz): δ 157.73, 154.75, 147.53, 145.31, 144.36, 142.91, 138.89, 135.59, 134.84, 134.31, 134.11, 133.27, 131.11, 129.24, 125.47, 124.56, 123.36. IR (KBr): 3442, 1700, 1507, 1217, 1038 cm^{-1}. MALDI TOF MS: m/z 1040 ($C_{44}H_{25}N_3Na_4O_{12}S_5$ + H, M + 1), 1018 ($C_{44}H_{26}N_3Na_3O_{12}S_5$ + H), 996 ($C_{44}H_{27}N_3Na_2O_{12}S_5$ + H), 974 ($C_{44}H_{28}N_3NaO_{12}S_5$ + H), 952 ($C_{44}H_{29}N_3O_{12}S_5$ + H). Anal. C, H, N, S.

Preparation of 5,10-Bis-4-sulfonatophenyl-15,20-bis-4-fluorophenyl-21-selena-23- thiaporphyrin Disodium Salt (9). Compound **5c** (0.21 g, 0.28 mmol) was treated as described to give 0.040 g (14%) of **9** as a metallic purple solid; mp >300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.24 (s, 2H), 9.88 (s, 2H), 8.91 (m, 4H), 8.51 (br s, 4H), 8.43 (AA′, J = 7 Hz), 8.34 (BB′, 4H, J = 7 Hz), 7.93 (t, 4H, J = 8 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.63 (d, J = 246 Hz, C−F), 157.12, 154.40, 151.93, 147.97, 144.44, 139.78, 138.31, 136.57, 135.70 (d, 8 Hz, C−C_2−F), 135.51, 135.28, 134.10, 133.51, 125.21, 114.76 (d, J = 22 Hz, C−C−F). IR (KBr): 3448 (br), 2364, 2345, 1223, 1187 cm^{-1}. High-resolution Q-TOF MS: m/z 936.9770 (calcd for $C_{44}H_{24}F_2N_2Na_2O_6S_3{}^{80}Se$ + H, 936.9801). Anal. C, H, N.

Preparation of 5,10-Bis-4-sulfonatophenyl-15,20-bis-4-fluorophenyl-21-thia-23-selenaporphyrin Disodium Salt (10). Compound **5d** (0.50 g, 0.69 mmol) was treated as described to give 0.40 g (67%) of **10** as a metallic purple solid; mp >300 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.20 (s, 2H), 9.90 (s, 2H), 8.91 (d, 4H, J = 4 Hz), 8.47 (m, 8H), 8.35 (d, 4H, J = 8 Hz), 7.93 (d, 4H, J = 8 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.54 (d, J = 246 Hz, C−F), 157.19, 154.29, 151.91, 148.17, 144.40, 140.35, 138.07, 136.07, 136.03, 135.70 (d, J = 7 Hz, C−C_2−F), 135.31, 134.37, 134.04, 133.49, 124.98, 115.00 (d, J = 22 Hz, C−C−F). IR (KBr): 3448 (br), 2364, 2345, 1223, 1187 cm^{-1}. High-resolution Q-TOF MS: m/z 936.9770 (calcd for $C_{44}H_{24}F_2N_2Na_2O_6S_3{}^{80}Se$ + H, 936.9801). Anal. C, H, N.

Preparation of 5,10-Bis-4-sulfonatophenyl-15,20-bis-4-fluorophenyl-21,23-diselenaporphyrin Disodium Salt (11). Compound **5b** (0.27 g, 0.35 mmol) was treated as described to give 0.15 g (43%) of **11** as a metallic purple solid; mp >300 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 9.60 (s, 2H), 9.57 (s, 2H), 8.40 (s, 4H), 8.22 (AA′, 4H, J = 7 Hz), 7.99 (BB′, 4H, J = 7 Hz), 7.91 (br s, 4H), 7.46 (t, 4H). ^{13}C NMR (CD_3OD, 75 MHz): δ 163.02 (d, J = 247 Hz), 157.73, 157.61, 151.05, 150.67, 146.37, 143.63, 138.10, 137.93, 137.63, 137.54, 137.05 (d, J = 8 Hz), 135.22, 135.09, 126.40, 115.71 (d, J = 22 Hz). IR (KBr): 3432 (br), 2365, 2345, 1126, 1040 cm^{-1}. MALDI TOF MS: m/z 984 ($C_{44}H_{24}F_2N_2Na_2O_6S_2{}^{80}Se_2$ + H, M + 1), 962 ($C_{44}H_{25}F_2N_2NaO_6S_2{}^{80}Se_2$ + H), 940 ($C_{44}H_{26}F_2N_2O_6S_2{}^{80}Se_2$ + H). Anal. C, H, N.

Preparation of 5,10-Bis-4-sulfonatophenyl-15,20-bis-4-fluorophenyl-21-thiaporphyrin Disodium Salt (12). 5,10-Bis-4-fluorophenyl-15,20-bisphenyl-21-thiaporphyrin (0.25 g, 0.37 mmol) was treated as described to give 0.15 g (46%) of **12** as a metallic purple solid; mp >300 °C. ^{1}H NMR (CD_3OD, 500 MHz): δ 9.56 (s, 2H), 8.89 (s, 2H), 8.49 (d, 2H, J = 5 Hz), 8.44 (d, 2H, J = 5 Hz), 8.18 (AA′, 4H, J = 8 Hz), 8.13 (BB′, 4H, J = 8 Hz), 7.99 (m, 4H), 7.40 (t, 4H, J = 8 Hz). ^{13}C NMR (CD_3OD, 75 MHz): δ 157.26 (d, J = 240 Hz, C−F), 148.60, 146.40, 145.31, 139.80, 138.01, 136.80, 136.69, 136.39, 135.52 (d, J = 7 Hz), 135.25, 134.15, 131.81, 130.14, 125.53, 124.11, 115.61 (d, J = 22 Hz, C−C−F). IR (KBr): 3448 (br), 2366, 1221, 1124, 1039 cm^{-1}. High-resolution Q-TOF MS: m/z 872.0767 (calcd for $C_{44}H_{25}F_2N_3Na_2O_6S_3$ + H, 872.0747). Anal. C, H, N.

Preparation of 5,10-Bis-4-sulfonatophenyl-15,20-bis-4-(trifluoromethyl)phenyl-21,23-dithiaporphyrin Disodium Salt (13). Compound **5e** (0.056 g, 0.71 mmol) was treated as described to give 0.040 g (56%) of **13** as a metallic purple solid; mp >300 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 9.68 (d, 4H, J = 9 Hz), 8.60 (m, 4H), 8.37 (AA′, 4 H, J = 8 Hz), 8.25 (s, 8H), 8.18 (BB′, 4H, J = 8 Hz). ^{13}C NMR (CD_3OD, 75 MHz): δ 180.42, 175.22, 157.78, 157.62, 149.21, 148.94, 146.47, 144.14, 143.89, 139.26, 136.97, 136.71, 135.75, 135.69, 135.40 (q, J = 8 HZ), 134.9 (q, J = 21 Hz), 129.50, 127.9 (q, J = 135 Hz), 126.36. IR (KBr): 3448 (br), 2367, 1578, 1396 cm^{-1}. MALDI TOF MS: m/z 889 ($C_{46}H_{24}F_6N_2Na_2O_6S_4$ + H, M + 1), 867 ($C_{46}H_{25}F_6N_2NaO_6S_4$ + H), 845 ($C_{46}H_{26}F_6N_2O_6S_4$ + H). Anal. C, H, N.

Preparation of 5,10-Bis-4-sulfonatophenyl-15,20-bis-4-*N*,*N*-(dimethylamino)phenyl)-21,23-dithiaporphyrin Disodium Salt (14). Compound **5f** (0.28 g, 0.38 mmol) was treated as described to give 0.15 g (42%) of **14** as a dark purple solid; mp >300 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 9.73 (s, 2H), 9.68 (s, 2H), 8.66 (d, 4H, J = 4 Hz), 8.59 (d, 4H, J = 4 Hz), 8.24 (AA′, 4H, J = Hz), 8.16 (BB′, 4H, J = 8 Hz), 7.98 (AA′, 4H, J = 9 Hz), 7.05 (BB′, 4H, J = 9 Hz), 3.07 (s, 12H). ^{13}C NMR (CD_3OD, 75 MHz): δ 157.87, 157.12, 151.43, 149.69, 148.14, 146.21, 144.39, 137.16, 136.78, 136.60, 136.06, 135.90, 134.99, 134.18, 133.12, 129.64, 126.30, 112.33, 40.38. High-resolution Q-TOF MS: m/z 939.1433 (calcd for $C_{48}H_{36}N_4Na_2O_6S_4$ + H, 939.1391). Anal. C, H, N.

Preparation of 5,10-bis-4-sulfonatophenyl-15,20-bis(2-sulfonato-4-methylphenyl)-21,23-dithiaporphyrin (15). 5,10-Bis(4-methylphenyl)-15,20-bis-phenyl-21,23-dithioporphyrin (0.46 g, 0.68 mmol) was treated as described and recrystallized from MeOH/CH_2Cl_2 to give 0.40 g (57%) of **15** as a metallic purple solid; mp >300 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 9.68 (s, 1H), 9.66 (s, 1H), 9.64 (s, 1H), 9.63 (s, 1H), 8.73 (d, 2H, J = 4 Hz), 8.58 (m, 4H), 8.25 (collapsed AA"BB", 8H), 8.11 (d, 1H, J = 7.5 Hz), 8.02 (d, 1H, J = 7.5 Hz), 7.69 (d, 1H, J = 8 Hz), 7.64 (d, 1H, J = 7.5 Hz), 2.94 (s, 6H). ^{13}C NMR (CD_3OD, 75 MHz): δ 157.89, 157.68, 149.35, 148.97, 146.48, 144.14, 144.08, 139.31, 138.19, 136.93, 136.84, 136.78, 135.74, 135.37, 135.06, 134.47, 133.71, 132.13, 126.36, 20.76. IR (KBr): 3458 (br), 2365, 2343, 1183, 1032. MALDI TOF MS: m/z 1085 ($C_{46}H_{28}N_2Na_4O_{12}S_6$ + H, M + 1), 1063 ($C_{46}H_{29}N_2Na_3O_{12}S_6$ + H), 1041 ($C_{46}H_{31}N_2Na_2O_{12}S_6$ + H), 1019 ($C_{46}H_{32}N_2NaO_{12}S_6$ + H), 997 ($C_{46}H_{32}N_2O_{12}S_6$ + H). Anal. C, H, N.

Quantum Yield Determinations. Quantum yields for singlet oxygen were measured in 0.01 M PBS at pH 7.4 with 1.6% NaCl at 25 °C using methods we have previously described.[22,24]

Cells and Culture Conditions. Colo-26, a murine colon carcinoma cell line, was maintained in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics (all components purchased from GIBCO Laboratories, Grand Island, NY) at 37 °C, 5% CO_2. R3230AC, a rat mammary adenocarcinoma

cell line, was maintained in minimum essential media supplemented with 10% FBS (Atlanta Biologicals, Atlanta, GA), 50 units/mL of penicillin G, 50 mg/mL of streptomycin, and 1.0 mg/mL of fungizone (MEM).

In Vitro Phototoxicity Measurements. Cells were plated at 5×10^3 cells/well of a 96 well tissue culture plate the evening before the assay. The day of the assay, the cells were washed twice with PBS, and 100 μL of HBSS containing various concentrations of photosensitizer was added to each well. The sensitizer and cells were incubated for 24 h at 37 °C followed by a wash with PBS and the addition of 100 μL of PBS. The plates were irradiated with filtered 570−800 nm light for a total light dose of 4 J cm^{-2}. Following irradiation, 100 μL of growth media was added and the plates were incubated for 24 h at 37 °C, 5% CO_2. Cell survival was monitored using the MTT assay as described in Mosmann.[25]

Animals. All animals were cared for under the guidelines of the Roswell Park Cancer Institute Committee on Animal Resources.

Photosensitizer Administration. For animal experimentation, the sensitizers were dissolved in saline or 5% dextrose in water (D5W). Injection was intravenous via the tail vein.

Fluorescence Measurement of Tissue Distribution of Sensitizers (Solvable Assay.) Tissue samples and tumors were harvested at various times posttreatment with **1** or **8** [5 mg (5 μmol)/kg] and flash frozen at −70 °C. Samples were thawed on ice, and 1 mL of Solvable (Packard Instrument Company, Meriden, CT) was added prior to incubation at 55 °C for 18−24 h. Samples were allowed to cool to ambient temperature, and the fluorescence per sample was determined and compared to a standard curve generated by the dilution of pure photosensitizer. Results are presented as total dye concentration per milligram of total protein. Total protein is measured using the BioRad protein assay (BioRad Laboratories).

PDT with 1, 8, 11, and Photofrin. Colo-26 tumors were implanted in BALB/c mice via the sterile trochar method. Sensitizer was administered 96 h following tumoring of the animals. Four hours following administration of sensitizer **1** [10 mg (10 μmol)/kg], **8** [0.125 mg (0.13 μmol)/kg], **11** [2.5 mg (2.5 μmol)/kg], or Photofrin [2.5 mg (4 μmol)/kg], the tumors were irradiated with red light at 630 nm at 75 mW cm^{-2} for 30 min for Photofrin or 694 nm at 75 mW cm^{-2} for 30 min for **1**, **8**, and **11** for a total light dose of 135 J cm^{-2}. Treated animals were followed until a tumor volume of 400 mm^3 was reached. An untreated control group received neither light nor drug.

Statistical Analyses. All statistical analyses were performed using the Student's *t*-test for pairwise comparisons. A P value of < 0.05 was considered significant.

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Supporting Information Available: NMR data for compounds **3**−**6**, Figure S1 for ambient and 60 °C ^{1}H NMR spectra of **15**, Figure S2 for the absorbance/emission spectrum of **8**, Figure S3 for the photobleaching of various sensitizers, Figures S4−S17 for the dark and phototoxicity of **1**, **2**, **7**−**17**, and Photofrin against the murine colon carcinoma cell line Colo-26, and Figure S18 for the phototoxicity of **8**, **9**, **11**, **14**, **17**, and Photofrin against R3230AC rat mammary adenocarcinoma cells. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. Photodynamic Therapy. *J. Natl. Cancer Inst.* **1998**, *90*, 889−905.

(2) Henderson, B. W.; Dougherty, T. J. How Does Photodynamic Therapy Work? *Photochem. Photobiol.* **1992**, *55*, 145−157.

(3) Kato, H. Photodynamic Therapy for Lung Cancer—A Review of 19 Years' Experience. *J. Photochem. Photobiol., B* **1998**, *42*, 96−99.

(4) Guillemin, F.; Feintrenie, X.; Lignon, D. Photodynamic Therapy of Bronchial Cancers. *Rev. Pneumologie Clin.* **1992**, *48*, 111−114.

(5) Puolakkainen, P.; Schroder, T. Photodynamic Therapy of Gastrointestinal Tumors: A Review. *Dig. Dis. Sci.* **1992**, *10*, 53−60.

(6) Noske, D. P.; Wolbers, J. G.; Sterenborg, H. J. Photodynamic Therapy of Malignant Glioma. A Review of Literature. *Clin. Neurol. Neurosurg.* **1991**, *93*, 293−307.

(7) Moesta, K. T.; Schlag, P.; Douglass, H. O., Jr.; Mang, T. S. Evaluating the Role of Photodynamic Therapy in the Management of Pancreatic Cancer. *Lasers Surg. Med.* **1995**, *16*, 84−92.

(8) Sharman, W. M.; Allen, C. M.; van Lier, J. E. Photodynamic Therapeutics: Basic Principles and Clinical Applications. *Drug Discovery Today* **1999**, *4*, 507−517.

(9) Bellnier, D. A.; Dougherty, T. J. A Preliminary Pharmacokinetic Study of Intravenous Photofrin in Patients. *J. Clin. Laser Med. Surg.* **1996**, *14*, 311−314.

(10) Dougherty, T. J.; Marcus, S. L. Photodynamic Therapy. *Eur. J. Cancer* **1992**, *28A*, 1734−1742.

(11) Stilts, C. E.; Nelen, M. I.; Hilmey, D. G.; Davies, S. R.; Gollnick, S. O.; Oseroff, A. R.; Gibson, S. L.; Hilf, R.; Detty, M. R. Water-Soluble, Core-Modified Porphyrins as Novel, Longer-Wavelength-Absorbing Sensitizers for Photodynamic Therapy. *J. Med. Chem.* **2000**, *43*, 2403−2410.

(12) (a) Berg, K.; Prydz, K.; Moan, J. Photochemical Treatment with the Lysosomally Localized Dye Tetra(4-sulfonatophenyl)porphine Results in Lysosomal Release of the Dye but not of β-N−Acetyl-D-glucosamidase Activity. *Biochim. Biophys. Acta* **1993**, *1158*, 300−306. (b) Kessel, D.; Thompson, P.; Saatio, K.; Nantwi, K. D. Tumor Localization and Photosensitization by Sulfonated Derivatives of Tetraphenylporphine. *Photochem. Photobiol.* **1987**, *45*, 787−790. (c) Kessel, D.; Woodburn, K. Biodistribution of Photosensitizing Agents. *Int. J. Biochem.* **1993**, *25*, 1377−1383. (d) Moan, J.; Iani, V.; Ma, L. W. In Vivo Fluorescence of Phthalocyanines During Light Exposure. *J. Photochem. Photobiol., B* **1998**, *42*, 100−103.

(13) (a) Sema, A. A. F.; Kennedy, J. C.; Blakeslee, D.; Robertson, D. M. Experimental Porphyric Neuropathy: A Preliminary Report. *Can. J. Neurol. Sci.* **1981**, *8*, 105−114. (b) Winkelman, J. W.; Collins, G. H. Neurotoxicity of Tetraphenylporphinesulfonate $TPPS_4$ and Its Relation to Photodynamic Therapy. *Photochem. Photobiol.* **1987**, *46*, 801−807.

(14) (a) Ulman, A.; Manassen, J. Synthesis of Tetraphenylporphyrin Molecules Containing Heteroatoms Other than Nitrogen. Part 4. Symmetrically and Unsymmetrically Substituted Tetraphenyl-21, 23-dithiaporphyrins. *J. Chem. Soc., Perkin Trans. 1* **1979**, *4*, 1066−1069. (b) Ulman, A.; Manassen, J.; Frolow, F.; Rabinovich, D. Synthesis of New Tetraphenylporphyrin Molecules Containing Heteroatoms Other than Nitrogen: II. Tetraphenyl-21-selena-23-thiaporphyrin and tetraphenyl-21, 23-diselenaporphyrin. *Tetrahedron Lett.* **1978**, 167−170. (c) Ulman, A.; Manassen, J.; Frolow, F.; Rabinovich, D. Synthesis of New Tetraphenylporphyrin Molecules Containing Heteroatoms other than Nitrogen. III. Tetraphenyl-21-tellura-23-thiaporphyrin: An Internally-Bridged Porphyrin. *Tetrahedron Lett.* **1978**, 1885−1886. (d) Jeyaprakash Narayanan, S.; Sridevi, B.; Chandrashekar, T. K.; Vij, A.; Roy, R. Novel Core-Modified Expanded Porphyrins with *meso*-Aryl Substituents; Synthesis, Spectral and Structural Characterization. *J. Am. Chem. Soc.* **1999**, *121*, 9053−9068.

(15) (a) Latos-Grazynski, L.; Lisowski, J.; Olmstead, M. M.; Balch, A. L. Five-Coordinate Complexes of 21-Thiaporphyrin. The Preparation, Spectra, and Structures of Iron(II), Nickel(II), and Copper(II) Complexes. *Inorg. Chem.* **1989**, *28*, 1183−1188. (b) Latos-Grazynski, L.; Pacholska, E.; Chmielewski, P. J.; Olmstead, M. M.; Balch, A. L. Synthesis of 5,20-Diphenyl-10, 15-bis(p-tolyl)-21-selenaporphyrin (SeDPDTPH). *Inorg. Chem.* **1996**, *35*, 566−573.

(16) (a) Ziolkowski, P.; Milach, J.; Symonowicz, K.; Chmielewski, P.; Latos-Grazynski, L.; Marcinkowska, E. 5,20-Bis(4-sulphophenyl)-10,15-bis(2-methoxy-4-sulfophenyl)-21-thiaporphyrin as a New Potent Sensitizer in Photodynamic Therapy. *Tumori* **1995**, *81*, 364−369. (b) Marcinkowska, E.; Ziolkowski, P.; Pacholska, E.; Latos-Grazynski, L.; Chmielewski, P.; Radzikowski, C. Z. The New Sensitizing Agents for Photodynamic Therapy: 21-Selenaporphyrin and 21-Thiaporphyrin. *Anticancer Res.* **1997**, *17*, 3313−3320.

(17) Meng, G. G.; James, B. R.; Skov, K. A.; Korbelik, M. Porphyrin Chemistry Pertaining to the Design of Anti-cancer Drugs; Part 2, the Synthesis and in Vitro Tests of Water-soluble Porphyrins

Containing in the *meso* Positions, the Functional Groups: 4-Methylpyridinium, 4-Sulfonatophenyl, in Combination with Phenyl, 4-Pyridyl, 4-Nitrophenyl, or 4-Aminophenyl. *Can. J. Chem.* **1994**, *71*, 2447−2457.

(18) Pandian, R.; Chandrashekar, T. K. Water-soluble Thiaporphyrins and Metallothiaporphyrins: Synthesis, Characterization, Ground- and Excited-state Properties. *J. Chem. Soc., Dalton Trans. 1* **1993**, 119−125.

(19) Wagnières, G. A.; Star, W. M.; Wilson, B. C. In Vivo Fluorescence Spectroscopy and Imaging for Oncological Applications. *Photochem. Photobiol.* **1998**, *68*, 603−632.

(20) (a) Braichotte, D. G.; Wagnières, R.; Bays, P.; Monnier, P.; van den Bergh, H. Clinical Pharmacokinetic Studies of Photofrin by Fluorescence Spectroscopy in the Oral Cavity, the Esophagus, and the Bronchi. *Cancer* **1995**, *75*, 2768−2778. (b) Wagnières, R.; Studzinski, A. P.; Braichotte, D. R.; Monnier, P.; Depeursinge, C.; Châtelain, A.; van den Bergh, H. E. Clinical Imaging Fluorescence Apparatus for the Endoscopic Photodetection of Early Cancers by Use of Photofrin-II. *Appl. Opt.* **1997**, *36*, 5608−5620.

(21) (a) Knyukshto, V. N.; Solovyov, K. N.; Egorova, G. D. Luminescence and Structure of the Protonated Forms of *meso*-Tetraarylporphyrins in Solution. *Biospectroscopy* **1998**, *4*, 121−133. (b) Micali, N.; Mallamace, F.; Romeo, A.; Purrello, R.; Monsù Scolaro, L. Mesoscopic Structure of *meso*-Tetrakis(4-sulfonatophenyl)porphine J-Aggregates *J. Phys. Chem. B* **2000**, *104*, 5897−5904. (c) Van Patten, P. G.; Shreve, A. P.; Donohoe, R. J. Structural and Photophysical Properties of a Water-soluble Porphyrin Associated with Polycations in Solution and Electrostatically assembles Ultrathin Films. *J. Phys. Chem. B* **2000**, *104*, 5986−5992.

(22) Detty, M. R.; Merkel, P. B. Chalcogenopyrylium Dyes as Potential Photochemotherapeutic Agents. III. Solution Studies of Heavy Atome Effects on Triplet Yields, Quantum Efficiencies of Singlet Oxygen Generation, Rates of Reaction with Singlet Oxygen, and Emission Quantum Yields. *J. Am. Chem. Soc.* **1990**, *112*, 3845−3851.

(23) Wilkinson, F.; Helman, W. P.; Ross, A. B. Quantum Yields for the Photosensitized Formation of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution. *J. Phys. Chem. Ref. Data* **1993**, *22*, 113−262.

(24) (a) Hawkins, C. W.; Bickers, D. R.; Mukhtar, H.; Elmets, C. A. Cutaneous Porphyrin Photosensitization: Murine Ear Swelling as a Marker of the Acute Response. *J. Invest. Dermatol.* **1986**, *86*, 638−642. (b) Bellnier, D. A.; Dougherty, T. J. The Time Course of Cutaneous Porphyrin Photosensitization of the Murine Ear. *Photochem. Photobiol.* **1989**, *49*, 369−372.

(25) Leonard, K. A.; Nelen, M. I.; Anderson, L. T.; Gibson, S. L.; Hilf, R.; Detty, M. R. 2,4,6-Triarylchalcogenopyrylium Dyes Related in Structure to the Antitumor Agent AA1 as in Vitro Sensitizers for the Photodynamic Therapy of Cancer. *J. Med. Chem.* **1999**, *42*, 3942−3952.

(26) Mosmann, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

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