In Vitro Photodynamic Properties of Chalcogenopyrylium Analogues of the Thiopyrylium Antitumor Agent AA1

Nancy K. Brennan,[†] Jonathan P. Hall,[†] Sherry R. Davies,[§] Sandra O. Gollnick,[§] Allan R. Oseroff,[§] Scott L. Gibson,[‡] Russell Hilf,[‡] and Michael R. Detty[†,*]

Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York 14260, Department of Biochemistry and Biophysics, University of Rochester Medical Center, 601 Elmwood Avenue, Box 607, Rochester, New York 14642, and Department of Dermatology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York 14263

Received June 19, 2002

Several series of chalcogenopyrylium dyes were prepared with one or two 4-anilino substituents at the 2- and 6-positions and with phenyl, 4-*N*,*N*-dimethylanilino, or 4-(*N*-morphilino)phenyl substituents at 2- and/or 4-positions. The dye series are all related in structure to **AA1**, a thiopyrylium dye that targets mitochondria. The chalcogenopyrylium nuclei included sulfur, selenium, and tellurium at the 1-position. Key intermediates in the dye synthesis were the corresponding Δ-4*H*-chalcogenopyran-4-ones. All of the dyes of this study were evaluated for dark and phototoxicity toward Colo-26 cells in vitro. There was no correlation of dark toxicity with either the reduction potential of the chalcogenopyrylium dye or the *n*-octanol/water partition coefficient, log *P*. Several of the dyes of this study (thiopyrylium dyes **1-S** and **13-S**, selenopyrylium dyes **1-Se**, **2-Se**, **3-Se**, **4-Se**, **13-Se**, **14-Se**, and **27-Se**, and telluropyrylium dye **13-Te**) showed added phototoxicity upon irradiation. Dyes with the highest therapeutic ratio as measured by dark toxicity/phototoxicity (15 J cm^{-2} of 360−800-nm light) had values of log *P* of 1.0−1.2. Studies of cytochrome *c* oxidase activity in whole R3230AC cells suggested that dyes **1-S** and **3-Se**, with values of log *P* of 2.2 and 1.7, respectively, were localized in the mitochondria. Cytocrome *c* oxidase activity in whole cells was inhibited by **1-S** and **3-Se** in the dark. Chalcogenopyrylium dyes **2-Se**, **4-Se**, **13-Te**, and **14-Se** inhibited whole-cell cytochrome *c* oxidase activity only following irradiation, which suggests that these dyes relocalized to mitochondria following irradiation.

Photodynamic therapy (PDT) as a protocol for treating cancer, as well as other diseases such as age-related macular degeneration, combines light and endogenous oxygen with a photosensitizer localized in the target tissue.[1] The photosensitizer is a critical component in PDT and a variety of molecular classes have been evaluated including porphyrins and related molecules, core-expanded porphyrins, core-modified porphyrins, chlorins and bacteriochlorins, and a variety of cationic dyes.[1] A photosensitizer should absorb wavelengths of light >600 nm, where penetration of light into tissue is optimal and should have a high quantum yield for the photochemical event that produces phototoxicity.[1] As with all drugs, adsorption, distribution, metabolism, and excretion (ADME) properties[2] of the photosensitizer are critical to the success of the technique. A photosensitizer with optimal ADME properties will have high specificity for the tumor or other target tissue, will undergo rapid distribution to the target tissue, will have minimal toxicity in the absence of light, and will be excreted following treatment to minimize any side effects such as long-term light sensitivity.

One significant difference between porphyrin photosensitizers and related molecules and cationic dyes is that the cationic dyes appear to be bound intracellularly.[3−9] Uptake and retention of lipophilic cations is greater in some carcinoma cells relative to normal cells.[3−9] Reports have demonstrated that the mitochondria[3,5−9] and lysosomes[4] are important targets for lipophilic cations. A recent study has demonstrated that lysosomal, nuclear, and mitochondrial localization of a series of methylene blue analogues is directly related to the *n*-octanol/water partition coefficient (log *P*).[10] Initially, the methylene blue derivatives were found to localize in lysosomes in vitro, but subsequently relocalized upon irradiation as a function of log *P*.[10] For methylene blue (log $P = -1.0$), relocalization to the nucleus was observed. For the *N*,*N*-diethyl analogue (log $P = 0.3$), no relocalization was observed, and the dye remained in the lysosome. For higher *N*,*N*-dialkyl analogues (log $P > 1.0$), relocalization to the mitochondria was observed upon irradiation.

The cationic thiopyrylium dye **AA1** (Chart 1) inhibits growth of the human colon carcinoma cell line CX-1 in vitro incubated with the dye in the dark prolongs survival of mice implanted with several different tumor lines.[9] Mitochondria appeared to be a target as determined by **AA1**-induced inhibition of mitochondrial AT-Pase activity. Although **AA1** appeared to be localized in tumors, no additional toxicity was observed after irradiation of tumors borne on animals injected with **AA1**. One concern with **AA1** and other lipophilic cations is the narrow therapeutic window between an effective treatment dose and the dose where **AA1** becomes lethal.[9]

* To whom correspondence should be addressed at: Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, NY 14260, Phone: (716) 645−6800, ext 2200, FAX: (716) 645-6963. e-mail: mdetty@acsu.buffalo.edu

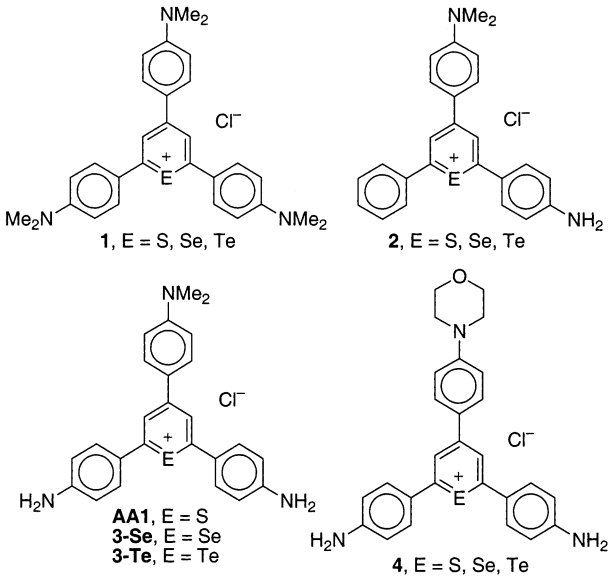
[†] Department of Chemistry, University at Buffalo.

[‡] Department of Biochemistry and Biophysics, University of Rochester Medical Center.

[§] Department of Dermatology, Roswell Park Cancer Institute.

10.1021/jm020260m CCC: $22.00
Published on Web 10/09/2002

Chart 1



We have been interested in analogues of **AA1** that show enhanced phototoxicity upon irradiation and offer promise as photosensitizers for PDT.[8] The enhanced phototoxicity of the cationic chalcogenopyrylium dyes would allow for a lower effective dose relative to the higher dose necessary for chemotherapy alone. The lower effective dose in PDT would expand the therapeutic ratio relative to a lethal dose. In our pursuit of **AA1**-like analogues, we have prepared and evaluated chalcogenopyrylium dyes **1**[8a] and **2**[8c] (Chart 1) and related compounds.[8b] Of these dyes, selenopyrylium dye **2-Se** was found to be an effective sensitizer for PDT against various carcinoma cell lines in culture and against R3230AC mammary adenocarcinomas implanted in female Fisher rats in vivo, where a 300% increase in tumor-doubling time was observed in treated animals relative to untreated controls.[8c] For dye classes **1** and **2**, the primary mechanism for phototoxicity appears to be the photochemical generation of singlet oxygen.[8]

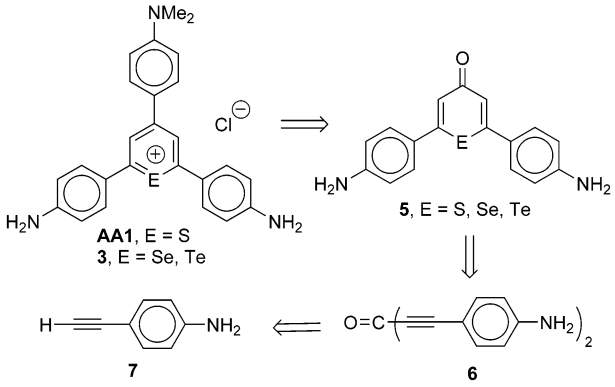
From the work with chalcogenopyrylium dyes **1** and **2** and related structures, the following generalizations can be made: (1) Analogues with the heavier chalcogen atoms selenium and tellurium give longer wavelengths of absorption and higher quantum yields for the photochemical generation of singlet oxygen than the corresponding sulfur analogues. (2) Two or three aniline-related substituents at the 2-, 4-, and 6-positions are required to give a photosensitizer. (3) The incorporation of one (**2-Se**)[8c] or two unsubstituted aniline groups (**AA1**)[9] gives desirable biodistribution relative to dyes **1** with three *N*,*N*-dimethylanilino substituents. Given these generalizations, we have investigated the effects of chalcogen atom substitution at the 1-position and substituent changes at the 2-, 4-, and 6-positions on measurable physical properties such as log *P* and the electrochemical reduction potential ($E°$) and correlated these properties with the dark and phototoxicity of the chalcogenopyrylium dyes toward cells in culture. Inhibition of cytochrome *c* oxidase activity in cultured whole cells exposed to the chalcogenopyrylium dyes and light suggests that the mitochondria are targets of localization or relocalization for the chalcogenopyrylium dyes.

We provide a general synthesis of selenopyrylium and telluropyrylium analogues of **AA1** with 4-anilino substituents at both the 2- and 6-positions as well as various combinations of phenyl and aniline-related substituents (4-anilino, 4-*N*-morpholinophenyl, and 4-*N*,*N*-dimethylanilino substituents).

Chemical Results and Discussion

Retrosynthetic Analysis and Synthesis. A. Selenopyrylium and Telluropyrylium Analogues of AA1. The chalcogenopyranones **5** are key intermediates for the synthesis of **AA1** and its selenopyrylium and telluropyrylium analogues as shown in the retrosynthesis of Scheme 1. The addition of Grignard reagents to the pyranone carbonyl followed by acid-induced dehydration will give the various chalcogenopyrylium analogues of **AA1**.[8c] Formally, the chalcogenopyranones **5** can be prepared by the 1,4-addition of a hydrogen chalcogenide to the triple bonds of 1,4-diynone **6**, which in turn can be prepared by addition of 2 equiv of an acetylide derived from *p*-aminophenylethyne **7** to a formate derivative followed by oxidation.

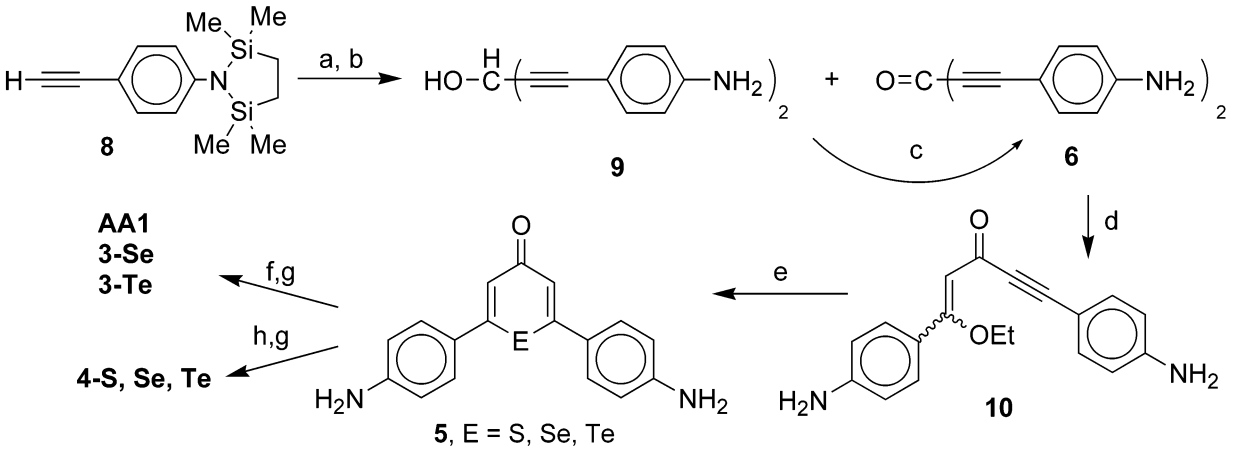
Scheme 1



Although acetylene **7** is known,[11] reactions of **7** with Grignard reagents and alkyllithium compounds are complicated by the free amino substituent. In view of our successful synthesis of dyes **2**, the amino group of **7** was protected with the bis-1,2-(dimethylsilyl)ethano (Stabase) protecting group[12] since this group is stable to strong bases under aprotic conditions and is easily hydrolyzed with either aqueous acid or base. The Stabase-protected acetylene **8** was deprotonated with *n*-BuLi in THF at −78 °C and 2 equiv of the resulting acetylide were added to a solution of methyl formate to give a mixture of 1,4-pentadiyn-3-ol **9** and 1,4-pentadiyn-3-one **6** (Scheme 2). The Stabase protecting group was lost during workup. Since the facile air oxidation of **9** to **6** made purification of **9** problematic, the crude product was oxidized directly with MnO_2 to give 1,4-pentadiyn-3-one **6** in 56% overall yield from **8**.

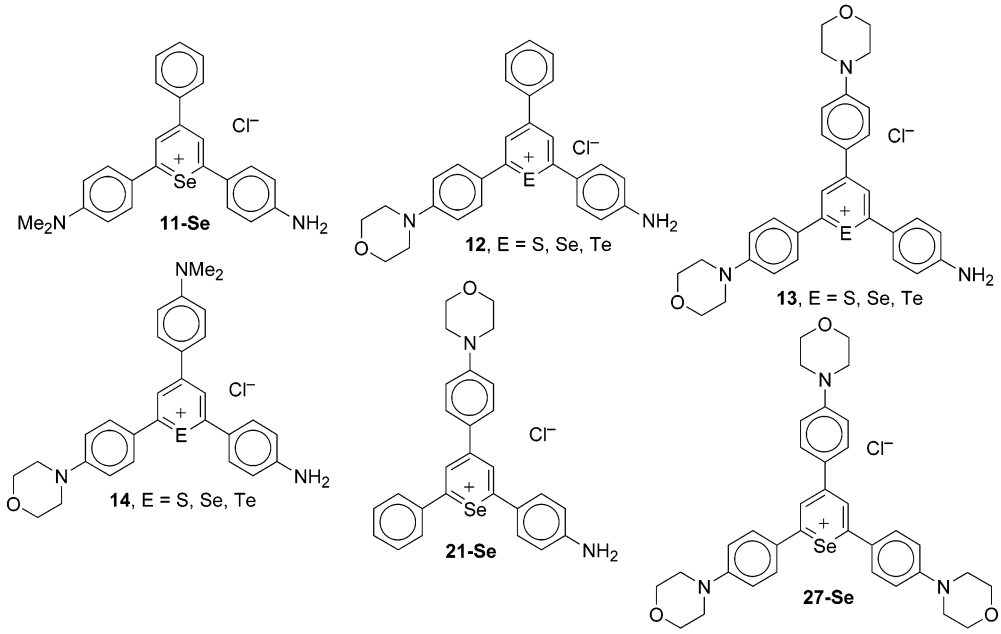
The preparation of chalcogenopyranones **5** via the formal addition of hydrogen chalcogenides to **6** was best accomplished in two steps to minimize 5-exo-trig cyclization to give dihydrochalcogenophene products.[13] Diynone **6** was stirred with 0.25 M NaOEt in EtOH for 2 h at ambient temperature to give enol ether **10** from addition of ethanol across one triple bond of **6** (Scheme

Scheme 2[a]



[a] Key: (a) *n*-BuLi, THF, −78 °C; (b) HCO_2Me; (c) MnO_2, CH_2Cl_2 (56% overall); (d) 0.25 M NaOEt in EtOH; (e) S, Se, Te, $NaBH_4$ (2.5 equiv), 0.1 M NaOEt in EtOH, add Na_2E to **10**; (f) *i*. $Me_2NC_6H_4MgBr$, *ii*. HPF_6; (g) Amberlite IRA-400 (Cl); (h) *i*. *N*-(4-bromophenyl)morpholine, Mg°, THF, *ii*. HPF_6, *iii*. Amberlite IRA-400 (Cl).

Chart 2

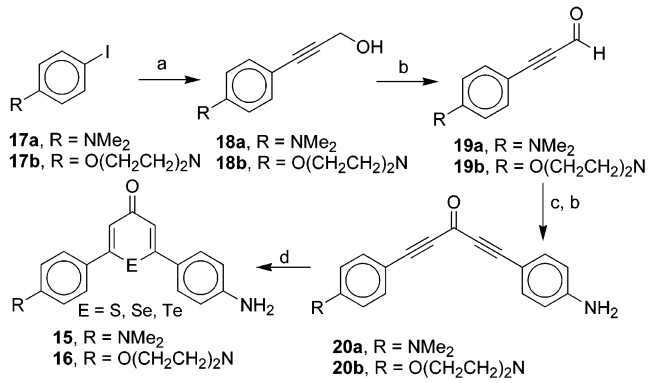


2). The addition of disodium chalcogenides to enol ether **10** gave chalcogenopyranones **5** in 21−57% isolated yields.

AA1 and its heavier chalcogenopyrylium analogues **3-Se** and **3-Te** were prepared by the addition of 4-dimethylaminophenylmagnesium bromide to chalcogenopyranones **5** followed by dehydration with HPF_6 (Scheme 2). Ion exchange with a chloride ion-exchange resin gave the chloride salts of the dyes. Similarly, the *N*-morpholinophenyl analogues **4** were prepared by the addition of the Grignard reagent derived from *N*-(4-bromophenyl)morpholine[14] followed by dehydration with HPF_6 and ion exchange to give the chloride salts **4** (Scheme 2).

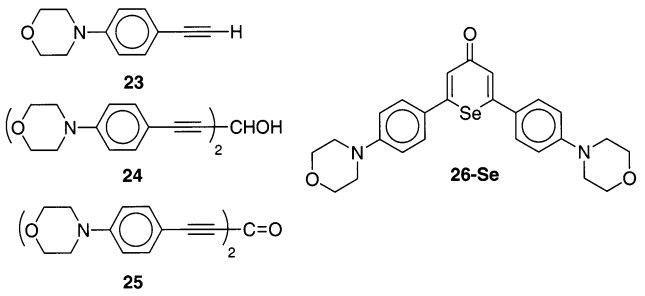
B. Chalcogenopyrylium Analogues with One 2-(4-Anilino) Substituent. The key intermediates to the preparation of dyes **11**−**14** (Chart 2), with one 4-anilino substituent, are the chalcogenopyranones **15** and **16**, which were prepared as shown in Scheme 3. Heck coupling of 4-iodo-*N*,*N*-dimethylaniline[15] (**17a**) or *N*-(4-iodophenyl)morpholine[16] (**17b**) with propargyl alcohol gave arylpropargyl alcohols **18a** and **18b** in 85% and 99% isolated yields, respectively.[17] The propargyl

Scheme 3[a]



[a] Key: (a) $PdCl_2(PPh_3)_2$, CuI_2, Et_3N, $HC{\equiv}CCH_2OH$; (b) MnO_2, CH_2Cl_2; (c) **8**, LDA, THF, −78 °C; (d) *i*. 0.25 M NaOEt, EtOH; *ii*. S, Se, or Te, $NaBH_4$ (2.5 equiv), 0.25 M NaOEt, EtOH, add Na_2E to *i*.

Chart 3



alcohols **18** were oxidized to the corresponding arylpropargyl aldehydes **19a**[15] and **19b** in 64% and 72% isolated yields, respectively, with MnO_2 in CH_2Cl_2.

The Stabase-protected[12] acetylene **8** was deprotonated with *n*-BuLi in THF at −78 °C, and 1 equiv of the resulting acetylide was added to a solution of propargyl aldehyde **19a** to give a mixture of 1,4-pentadiyn-3-ol and 1,4-pentadiyn-3-one **20a** (Scheme 3). The Stabase protecting group was lost during workup. The crude product mixture was oxidized with MnO_2 in CH_2Cl_2 to give 1,4-pentadiynone **20a** in 83% isolated yield. The same procedure was applied to propargyl aldehyde **19b** to give 1,4-pentadiyn-3-one **20b** in 31% overall yield.

As before, the preparation of chalcogenopyranones **15** and **16** via the formal addition of hydrogen chalcogenides to **20** was best accomplished in two steps. Diynone **20** was stirred with 0.25 M NaOEt in EtOH for 2 h at ambient temperature followed by the addition of disodium chalcogenides to give selenopyranone **15-Se** in 76% isolated yield and chalcogenopyranones **16** in 41−45% isolated yields.

The addition of phenylmagnesium bromide to selenopyranone **15-Se** and to chalcogenopyranones **16** followed by dehydration with HPF_6 gave the hexafluorophosphate salts of selenopyrylium dye **11-Se** and chalcogenopyrylium dyes **12**, respectively (Chart 2). The addition of the Grignard reagent derived from *N*-(4-bromophenyl)morpholine[14] to chalcogenopyranones **16** followed by dehydration with HPF_6 gave the hexafluorophosphate salts of dyes **13** (Chart 2). The addition of 4-dimethylaminophenylmagnesium bromide to chalcogenopyranones **16** followed by dehydration with HPF_6 gave the hexafluorophosphate salts of chalcogenopyrylium dyes **14** (Chart 2). The hexafluorophosphate salts of **11**−**14** were converted to the chloride salts using an ion-exchange resin.

Dye **21-Se** was prepared by the addition of the Grignard reagent derived from *N*-(4-bromophenyl)-morpholine[14] to Δ-4*H*-2-(4-anilino)-6-phenylselenopyran-4-one (**22**)[8c] followed by dehydration with HPF_6 to give the hexafluorophosphate salt of dye **21-Se** (Chart 2). As before, ion exchange gave the chloride salt of **21-Se**.

C. 2,4,6-Tris-4-(*N*-morpholino)phenyl Selenopyrylium Dye 27-Se. Propargyl aldehyde **19b** was treated with KOH to give *N*-ethynylmorpholine (**23**) in 97% isolated yield (Chart 3).[17] Acetylene **23** was treated with *n*-BuLi, and the resulting acetylide was added to propargyl aldehyde **19b** to give diynol **24** in 83% isolated yield. Diynol **24** was oxidized to diynone **25** in 83% isolated yield with MnO_2. Diynone **25** was stirred with 0.25 M NaOEt in EtOH for 2 h at ambient temperature followed by the addition of disodium selenide to give the key intermediate, selenopyranone **26-Se**, in 57% isolated yield. The addition of the Grignard reagent derived from *N*-(4-bromophenyl)morpholine[17] to chalcogenopyranone **26-Se** followed by dehydration with HPF_6 gave the hexafluorophosphate salt of dye **27-Se** in 82% isolated yield, which was then converted to the corresponding chloride salt with an ion-exchange resin (Chart 2).

Spectral and Physical Properties. A. Absorption Spectra. Useful photosensitizers have some absorption at wavelengths >600 nm, where penetration of light in tissue is maximal.[18] All of the dyes **3**, **4**, and **11**−**14** have some absorption at wavelengths >600 nm (values of λ_{max} and log ϵ are compiled in Table 1). For comparison purposes, values of λ_{max} and log ϵ are also compiled in Table 1 for dyes **1**[8a] and **2**.[8c] As with other series of chalcogenopyrylium dyes, the absorption maxima increase in wavelength as the heteroatom increases in size. Dye **11-Se** and dye series **12**, both with anilino derivatives at the 2- and 6-positions and a phenyl substituent at the 4-position, display large bathochromic shifts relative to dyes **2** with anilino derivatives at the 2- and 4-positions and a phenyl substituent at the 6-position and relative to dyes **1**, **13**, **14**, and **27-Se** with aniline related substituents at the 2-, 4-, and 6-positions. Replacing an *N*,*N*-dimethylanilino substituent with a 4-*N*-morpholinophenyl substituent gives hypsochromic shifts in absorption maxima as noted in comparisons of dyes **3** and **AA1** with dye series **4**, **11-Se** with **12-Se**, dye series **13** with **14**, and **27-Se** with **1-Se**.

Dyes **AA1**, **1-4**, **11**−**14**, **21-Se**, and **27-Se** all display a large hypsochromic shift as the solvent is changed from CH_2Cl_2 to water. Hydrogen-bond donation from the anilino group(s) of dyes **AA1**, **3**, **4**, **11**−**14**, and **21-Se** to water decreases the effective cationic charge on the chromophore and the corresponding cyanine character. Similarly, hydrogen bonding from water to the nitrogen atoms of the various anilino groups of dyes **AA1**, **1**−**4**, **11**−**14**, **21-Se**, and **27-Se** decreases the availability of the nitrogen lone-pair of electrons to the delocalized π-system.

B. Electrochemical Reduction Potentials. With the mitochondria as one possible target of the cationic chalcogenopyrylium dyes, one must be concerned with dark toxicity from disruption of the redox cascade in mitochondrial respiration. The cationic chalcogenopyrylium dyes are electron acceptors and electron transfer within the mitochondria to reduce the chalcogenopyrylium cation to the neutral chalcogenopyranyl radical is possible along with the possibility of disruption of mitochondrial function. The reduction potentials of **AA1**, dyes **1**−**4**, **11**−**14**, **21-Se**, and **27-Se** were determined by cyclic voltammetry and values of $E°$ for the cation/neutral radical couple are compiled in Table 1. Values of $E°$ for **AA1**, **1**−**4**, **11**−**14**, **21-Se**, and **27-Se** cover a 0.38-V range from −0.38 V for **12-Te** to −0.76 V for **1-S** [vs the ferrocene/ferrocinium couple (Fc/Fc^+) at +0.40 V]. Within each series of dyes, an anodic (positive) shift in $E°$ is observed as the chalcogen atom becomes heavier.

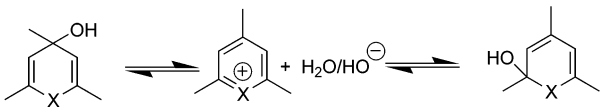
C. *n*-Octanol/Water Partition Coefficients. The determination of *n*-octanol/water partition coefficients for the chalcogenopyrylium dyes is complicated by the

Table 1. Absorption Maxima in CH_2Cl_2 and Water, Electrochemical Reduction Potentials ($E°$), *n*-Octanol/Water Partition Coefficients (log *P*), Dark Toxicity (LD_{50}), and Phototoxicity (EC_{50}) toward Colo-26 Cells in Culture, and Therapeutic Ratio (LD_{50}/EC_{50}) for Chalcogenopyrylium Dyes AA1, 1-4, 11-14, 21-Se, and 27-Se

					Colo-26 cells		
compd	λ_{max}, nm (log ϵ) CH_2Cl_2	λ_{max}, nm (log ϵ) H_2O	$E°$, V[a]	log *P*[b]	LD_{50} (μM)	EC_{50} (μM)	LD_{50}/EC_{50}
AA1	581 (4.74)	541 (4.40)	−0.71	1.9	0.1	-	-
1-S	620 (4.76)	595 (4.41)	−0.76	2.2	0.5	0.2	2.5
1-Se	655 (4.50)	627 (4.40)	−0.71	2.2	0.5	0.3	1.7
2-S	620 (4.49)	573 (4.45)	−0.60	1.3	0.07	-	-
2-Se	651 (4.47)	615 (4.40)	−0.51	1.2	2.6	0.2	13
2-Te	677 (4.50)	640 (4.40)	−0.44	1.9	0.6	-	-
3-Se	610 (4.84)	554 (4.51)	−0.61	1.7	1.4	0.37	3.8
3-Te	620 (4.60)	581 (4.42)	−0.55	2.0	0.5	-	-
4-S	566 (4.66)	535 (4.54)	−0.68	1.8	0.8	-	-
4-Se	593 (4.65)	553 (4.50)	−0.57	1.5	3.8	1.2	3.2
4-Te	618 (4.73)	582 (4.36)	−0.51	1.9	1.4	-	-
11-Se	670 (4.71)	635 (4.40)	−0.46	1.3	0.4	-	-
12-S	616 (4.64)	597 (4.32)	−0.49	1.1	1.8	-	-
12-Se	-645 (4.69)	626 (4.45)	−0.42	0.9	0.9	-	-
12-Te	674 (4.70)	653 (4.41)	−0.38	1.0	0.07	-	-
13-S	578 (4.84)	546 (4.28)	−0.58	1.3	66	2.0	33
13-Se	608 (4.71)	566 (4.36)	−0.52	1.2	6.9	1.9	3.6
13-Te	638 (4.54)	584 (4.38)	−0.41	1.6	0.8	0.6	1.3
14-S	589 (4.89)	553 (4.19)	−0.66	0.9	0.9	-	-
14-Se	616 (4.72)	557 (4.52)	−0.58	1.0	1.5	0.07	21
14-Te	646 (4.86)	583 (4.55)	−0.49	1.0	0.5	-	-
21-Se	611 (4.74)	580 (4.38)	−0.47	1.2	6.5	-	-
27-Se	622 (4.83)	582 (4.25)	−0.74	1.2	7.2	0.8	9.0

[a] In CH_2Cl_2 with 0.2 N Bu_4NBF_4 as supporting electrolyte, V vs the ferrocene/ferricinium couple ($E° = +0.40$ V). [b] pH-6 Phosphate buffer.

Scheme 4



addition of H_2O/HO^- to the chalcogenopyrylium nucleus at either the 2- or 4-position as shown in Scheme 4.[8a,19] When the chalcogenopyrylium dye is partitioned between *n*-octanol and water, hydrolysis is more rapid in the aqueous phase than in the organic phase. Apparent values of log *P* are influenced by the rate of diffusion between layers as well as the rate and extent of addition of H_2O/HO^-. At physiological pH (7.4), apparent values of log *P* can vary significantly (±0.7 units in log *P*). We have found that minimal addition of H_2O/HO^- occurs at pH 6 and that values of log *P* can be more reproducibly determined (±0.1 units of log *P*) using a mixture of *n*-octanol and pH 6 phosphate buffer.

Values of log *P* were determined for **AA1**, **1**−**4**, **11**−**14**, **21-Se**, and **27-Se** using a pH 6 phosphate buffer as the aqueous phase via the "skake flask" direct measurement.[20] Values of log *P* are compiled in Table 1 and cover a range of 0.9 to 2.2.

Biological Results and Discussion

In Vitro Studies. Chalcogenopyrylium dyes **AA1**, **1**−**4**, **11**−**14**, **21-Se**, and **27-Se** were evaluated in culture for dark or light-induced toxicity toward Colo-26 cells, a murine colon carcinoma cell line. Cell cultures were incubated for 24 h in the dark with various concentrations of cationic dyes and were then washed prior to treatment with filtered 360−800-nm light from a tungsten−halogen source for a total light dose of 15 J cm^{-2}. All of the chalcogenopyrylium dyes had absorption bands with comparable extinction coefficients and bandwidths at half-height in this window. Light-treated cells and dark controls were incubated for 24 h and cell survival was determined. Results are reported in Table 1 for the LD_{50} for each dye with respect to dark toxicity and, for those examples in which added phototoxicity was observed, the effective concentration (EC_{50}) to give 50% cell kill 24 h following irradiation with 15 J cm^{-2} of 360−800-nm light.

A. Dark Toxicity toward Colo-26 Cells in Culture. With common substituents at the 2-, 4-, and 6-positions, the ring heteroatom had a significant impact on the observed dark toxicity toward cultured Colo-26 cells in vitro (Table 1). For dye series **14**, the dark toxicity varied by a factor of 3 for the three chalcogen atoms; for dye series **4**, by a factor of 4.8; for the series of **AA1** and dyes **3**, by a factor of 14; for dye series **12**, by a factor of 26; for dye series **2**, by a factor of 37; and for dye series **13**, by a factor >50. For the series of **AA1**, **3-Se**, and **3-Te** and related series **2** and **4**, the thiopyrylium analogues **AA1**, **2-S**, and **4-S** displayed the greatest dark toxicity while the selenopyrylium analogues **2-Se**, **3-Se**, and **4-Se** displayed the least. In dye series **12**−**14**, the telluropyrylium analogues displayed the greatest dark toxicity.

The substituents at the 2-, 4-, and 6-positions also impacted the observed dark toxicity toward Colo-26 cells in vitro. One general observation of the effect of substituents at the 2-, 4-, and 6-positions of the chalcogenopyrylium dyes is that replacing a phenyl, 4-*N*,*N*-dimethylanilino, or a 4-anilino substituent with a 4-*N*-morpholinophenyl substituent resulted in a decrease in dark toxicity (Table 1).

The changes in dark toxicity associated with changes in either the ring heteroatom or in the 2-, 4-, and 6-substituents did not correlate with any systematic changes in either reduction potential, $E°$, or *n*-octanol/water partition coefficient, log *P*. As shown in Figure S1 (Supporting Information), there is no correlation of a 10^3 range in dark toxicity with a 0.38-V range in

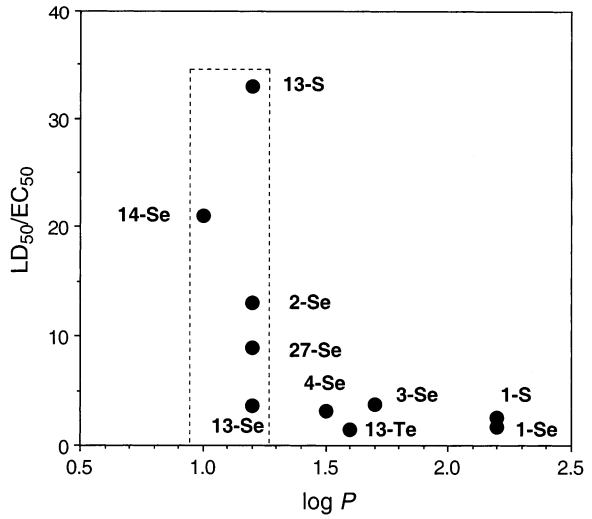


Figure 1. A plot of the therapeutic ratio between dark toxicity and phototoxicity (LD_{50}/EC_{50}) as a function of the *n*-octanol/water partition coefficient (log *P*) for dyes **1-S**, **13-S**, **1-Se**, **2-Se**, **3-Se**, **4-Se**, **13-Se**, **13-Te**, **14-Se**, and **27-Se**.

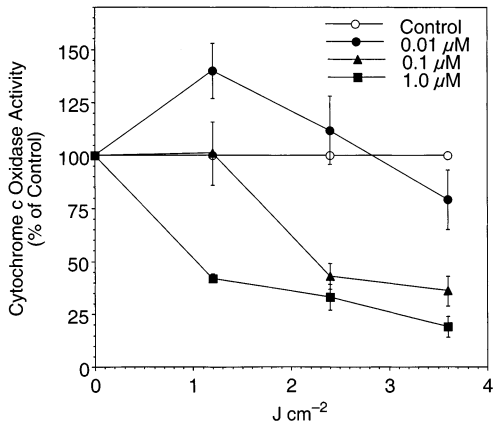


Figure 2. Effect of **2-Se** photosensitization on mitochondrial cytochrome *c* oxidase activity in cultured R3230AC tumor cells. Details of experimental conditions are described in the Experimental Section. Data are expressed as percentage of control cytochrome *c* oxidase activity, obtained from cells not exposed to either **2-Se** or light (○) and in cells exposed to 1.0×10^{-8} M (●), 1.0×10^{-7} M (▲) or 1.0×10^{-6} M (■) **2-Se** and light (0.5 mW/cm^2 of 360−750-nm light). Each datum point represents the mean of at least three separate experiments performed in duplicate, the error bars are the SEM.

reduction potential. As shown in Figure S2 (Supporting Information), there is no correlation of a 10^3 range in dark toxicity with a range of values of log *P* from 0.9 to 2.2.

B. Phototoxicity toward Colo-26 Cells in Culture. All of the chalcogenopyrylium dyes **AA1**, **1**−**4**, **11**−**14**, **21-Se**, and **27-Se** were examined for phototoxicity against cultured Colo-26 cells. Phototoxicity was observed with thiopyrylium dyes **1-S** and **13-S**, with selenopyrylium dyes **1-Se**, **2-Se**, **3-Se**, **4-Se**, **13-Se**, **14-Se**, and **27-Se**, and with telluropyrylium dye **13-Te**. Values of the effective concentration to give 50-% cell kill (EC_{50}) with 15 J cm^{-2} of 360−800-nm light are compiled in Table 1. The range of EC_{50} values varied from 0.07 μM for dye **14-Se** to 2 μM for dye **13-S**.

One trend observed in the phototoxicity studies was that derivatives with a 4-phenyl substituent displayed no increased toxicity upon irradiation compared to cells exposed to dyes in the dark. Compound **2-Se** proved to be an efficient photosensitizer of Colo-26 cells in vitro. If the 4-*N*,*N*-dimethylanilino and 6-phenyl substituents are interchanged, one obtains **11-Se**. Surprisingly, selenopyrylium dye **11-Se** displays no added phototoxicity. Similarly, none of the 4-phenyl-substituted derivatives **12** displays any greater toxicity upon irradiation than cells incubated with **12** in the dark.

The ratio of dark toxicity to phototoxicity as an approximation of the therapeutic ratio for the photosensitizers is perhaps a better measure of photosensitizer effectiveness. Values of LD_{50}/EC_{50} are compiled in Table 1 and cover a range from 1.3 for **13-Te** to 33 for **13-S**. The series of photosensitizers **1-S**, **1-Se**, **2-Se**, **3-Se**, **4-Se**, **13-S**, **13-Se**, **13-Te**, **14-Se**, and **27-Se** cover the 0.9−2.2 range of values of log *P*. In Figure 1, LD_{50}/EC_{50} is plotted as a function of log *P*. The data of Figure 1 suggest that the thiopyrylium and selenopyrylium sensitizers with the largest therapeutic ratios are clustered around log *P* values of 1.0−1.2.

C. Mitochondrial Localization or Relocalization. Cytochrome *c* Oxidase Activity in Whole Cells. The relocalization of a series of methylene blue photosensitizers from lysosomes to mitochondria or DNA upon irradiation was followed by observing fluorescence from the photosensitizer.[10] Unfortunately, the dyes studied in this present report are not fluorescent, which makes detection within cultured cells difficult. Furthermore, the heavy atoms present in the selenopyrylium and telluropyrylium analogues can quench fluorescence, which can complicate studies using fluorescent organelle tags. However, the mitochondria were identified as sites of localization or as sites of relocalization (following irradiation) due to changes in mitochondrial cytochrome *c* oxidase activity in whole cells treated with chalcogenopyrylium dyes and light.[8c]

Cytochrome *c* oxidase is a multisubunit complex, which spans the inner membrane of the mitochondria and which contains four one-electron redox centers including two copper ions and two heme units.[21] It is the last enzyme in the mitochondrial respiration chain, and loss of enzymatic activity is due to direct damage to this enzyme or to other sites preceding it in the respiration chain.

Dye **2-Se** has demonstrated efficacy in vivo[8c] and is one of the more potent photosensitizers in this study based on EC_{50} as well as the therapeutic ratio, LD_{50}/EC_{50} (Table 1). Dye **2-Se** also has a value of log *P* of 1.2, which is in the optimal range suggested by Figure 1. R3230AC rat mammary adenocarcinoma cells were incubated with 0.01−1.0 μM concentrations of **2-Se** for 24 h, and cultures were washed once with 0.9% NaCl and were then irradiated for various times up to 30 min with 360-to-750-nm light from a tungsten−halogen source delivered at 0.5 mW/cm^2. Immediately following irradiation, the cells were removed from the substrate and the activity of cytochrome *c* oxidase was determined in these samples. The data presented in Figure 2 demonstrate that mitochondrial cytochrome *c* oxidase activity in whole cells treated with **2-Se** and light is inhibited in a concentration-and-light-dose-dependent manner. Cells treated with **2-Se** alone or light alone showed no inhibition of cytochrome *c* oxidase compared to untreated control cells under these conditions. Fur-

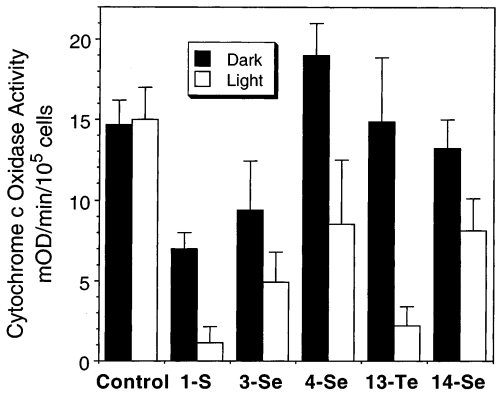


Figure 3. Effect of **1-S**, **3-Se**, **4-Se**, **13-Te**, or **14-Se** photosensitization on mitochondrial cytochrome *c* oxidase activity in cultured R3230AC tumor cells. Data are cytochrome *c* oxidase activity expressed as the change in optical density at 550 nm measured as mOD units/min/1 × 10^5 cells. A control group of cells received neither dye nor light. Each experimental group of cells was exposed to dye for 24 h in the dark and a second group was irradiated for 1 h with 360−750-nm light at 0.5 mW cm^{-2} (1.8 J cm^{-2}) followed by incubation in the dark for 24 h. Details of experimental conditions are described in the Experimental Section. Each datum point represents the mean of at least three separate experiments performed in duplicate; the error bars are the standard error from the mean (SEM).

thermore, inhibition of cytochrome *c* oxidase was observed with as little as 0.01 μM **2-Se**, which strongly suggests that the mitochondria are sites of localization or relocalization upon irradiation of cultured cells treated with **2-Se**.

The effects of dye and light treatment on whole cell cytochrome *c* oxidase activity with dyes **1-S**, **3-Se**, **4-Se**, **13-Te**, and **14-Se** was evaluated after a 24-hr incubation period. These dyes were selected to cover all of the chalcogen modifications, representative examples of substituent changes, and a range of values of log *P*. Cultured R3230AC rat mammary tumor cells were incubated with a 1.0 μM solution of **1-S** (log *P* 2.2), **3-Se** (log *P* 1.7), **4-Se** (log *P* 1.5), **13-Te** (log *P* 1.6), or **14-Se** (log *P* 1.0) for 24 hr**.** Cultures were washed once with 0.9% NaCl and irradiated for 1 h with 360-to-750-nm light from a tungsten−halogen source delivered at 0.5 mW/cm^2 (1.8 J cm^{-2}). Immediately following irradiation, the saline was removed from the substrate and replaced with media (MEM + 10% FBS). Both irradiated dye-treated cells and dye-treated dark controls were incubated for 24 h in the dark, and the activity of cytochrome *c* oxidase was determined in dye-treated irradiated cells and dye-treated dark controls (Figure 3). The data presented in Figure 3 demonstrate that mitochondrial cytochrome *c* oxidase activity in whole cells treated with **1-S**, **3-Se**, **4-Se**, **13-Te**, or **14-Se** and light is inhibited relative to untreated control cells and control cells treated with light only. Cells treated with **4-Se**, **13-Te**, or **14-Se** and no light did not inhibit mitochondrial cytochrome *c* oxidase activity while dyes **1-S** and **3-Se** displayed some inhibition of mitochondrial cytochrome *c* oxidase in the dark. These data and the results with **2-Se** suggest that mitochondria may be initial sites of localization or relocalization of chalcogenopyrylium dyes following irradiation. Upon irradiation, photodamage occurs at the mitochondria as evidenced by the inhibition of cytochrome *c* oxidase.

Summary and Conclusions

General synthetic routes to selenopyrylium and telluropyrylium analogues of **AA1** and to chalcogenopyrylium analogues of a number of closely related structures have been described. Several of these chalcogenopyrylium dyes are photosensitizers in vitro as determined by phototoxicity studies in cultured Colo-26 cells and whole cell mitochondrial cytochrome *c* oxidase studies performed in cultured R3230AC cells. In general, the selenopyrylium analogue in a series of chalcogenopyrylium dyes is most likely to show phototoxicity due to the heavy atom effect increasing singlet oxygen yield,[8] although all three chalcogen analogues of dye series **13** demonstrated some phototoxicity.

While phototoxicity was observed for the chalcogenopyrylium dyes in this study for a range of log *P* values from 1.0 to 2.2, the highest therapeutic ratio (LD_{50}/EC_{50}) was found for dyes with log *P* values of roughly from 1.0 to 1.2 (Figure 1). The chalcogenapyrylium dyes in this range include the more phototoxic compounds (**2-Se** with EC_{50} of 0.2 μM, **14-Se** with EC_{50} of 0.07 μM) as well as compounds that are phototoxic at higher concentrations such as **13-S** which has an EC_{50} of 2.0 μM. Over the range of log *P* values from 0.9 to 2.2, there is no apparent correlation between dark toxicity and log *P* (Figure S2, Supporting Information). However, compounds with log *P* values ranging from 1.0 to 1.2 appear to be more effective photosensitizers of cultured Colo 26 cells. This dependency on a range of log *P*, hydrophobicity/hydrophilicity, for photosensitizer efficacy has been reported for other dye classes such as Nile blue derivatives,[22] porphyrins with a variety of side chain substituents,[23] and methylene blue analogues.[10]

At this time, we cannot define the site(s) of localization of the chalcogenopyrylium dyes, but the mitochondria appear to be one site of photodamage. On the basis of studies with rhodamines,[3] nile blue derivatives,[4] and methylene blue derivatives,[10] it is likely that the chalcogenopyrylium dyes are concentrated initially in both the lysosomes and/or the mitochondria. The lipophilicity of the individual cations perhaps determines the relative concentration in the two sites. For the dyes with values of log *P* near the 1.0−1.2 range, initial localization may be in the lysozomes. Irradiation may release dyes from the lysosomes to concentrate in the mitochondria where photodamage occurs, therefore these dyes may show less dark toxicity intrinsically because damage may only occur at the lysosomes prior to irradiation. Relocalization of photosensitizers within the cell following irradiation has been reported for Nile blue derivatives[22] and *meso*-tetraphenylporphyrins.[23] Other chalcogenopyrylium dyes with higher values of log *P* may initially concentrate to a higher degree in the mitochondria and dark toxicity may be observed because of the probability that the chalcogenapyrylium dyes may disrupt mitochondrial function as indicated below. Dyes **1-S** and **3-Se**, with values of log *P* of 2.2 and 1.7, respectively, inhibit cytocrome *c* oxidase activity in whole cells in the dark, which is consistent with mitochondrial localization. Localization of lipophilic cationic dyes to the mitochondria of whole cells may also be due to both the plasma membrane potential and the mitochondrial electrochemical gradient.[9] These cellular properties

appear to cause the lipophilic cationic dyes to concentrate intracellulary 100−10000-fold compared to the concentration of the dye in the culture medium.[9] In this report, the mitochondria are damaged upon irradiation of dye-treated cells as evidenced by the light-induced inhibition of mitochondrial cytochrome *c* oxidase (Figure 3). Studies of the effects of the chalcogenapyrylium dyes on this enzyme support earlier conclusions that lipophilic cationic dyes initially localize or relocalize upon irradiation to the mitochondria of cultured cells.

The chalcogenopyrylium dyes are electron acceptors as measured by $E°$ for the cation/neutral radical redox couple, but there is no overall correlation of $E°$ with dark toxicity in cultured cells. However, dyes **1-S** and **3-Se**, with higher values of log *P* in this study (2.2 and 1.7, respectively) show some inhibition of whole-cell, mitochondrial cytochrome *c* oxidase activity in the dark, which may involve electron transfer to the chalcogenopyrylium dye.

Whether damage to the mitochondria in the dark or after irradiation in the presence of chalcogenapyrylium dyes is the underlying cause for loss in cell viability is not yet known. One aspect that is of interest is whether members of this class of photosensitizers are cytotoxic via induction of necrosis or through apoptosis. One could surmise that compounds with a high affinity for mitochondria might act on this organelle which is the source of cytochrome *c*, the factor, which when released from the mitochondria activates the apoptotic machinery in the cell leading to cell death. That this class of dyes may be involved in apoptosis is the focus of future studies in our laboratories.

Experimental Section

General Methods. Solvents and reagents were used as received from Sigma-Aldrich Chemical Co (St. Louis, MO) unless otherwise noted. Concentration in vacuo was performed on a Büchi rotary evaporator. Selenopyranone **22** was prepared according to ref 8c. NMR spectra were recorded on a Varian Gemini-300, Inova 400, or Inova 500 instruments with residual solvent signal as 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 equipped with a circulating constant-temperature bath for the sample chambers. Elemental analyses were conducted by Atlantic Microanalytical, Inc.

2,6-(4-Anilino)-4-(4-*N*,*N*-dimethylanilino)thiopyrylium Chloride (AA1).[9] A solution of Δ-4*H*-2,6-bis(4-anilino)-thiopyran-4-one (**5-S**, 0.050 g, 0.17 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *p*-bromo-*N*,*N*-dimethylaniline, (0.34 g, 1.7 mmol) and Mg turnings, (0.08 g, 3.4 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h, allowed to cool to ambient temperature and poured into CH_3CO_2H (3.0 mL). A 60% weight solution of HPF_6 in water was added dropwise until a color change was observed. Water (10 mL) was added, and the resulting solution was cooled to −10 °C. The precipitate was collected by filtration, and the solid was washed with water (10 mL) and ether (10 mL). The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.032 g (34%) the PF_6 salt of **AA1** as a dark blue solid, mp > 260 °C.

The hexafluorophosphate salt (0.020 g) was dissolved in 10 mL of CH_3CN, and 0.125 g of Amberlite IRA-400 Chloride ion-exchange resin was added. The resulting mixture was stirred 0.5 h, followed by removal of the exchange resin via filtration. The process was repeated with two additional 0.125-g aliquots of the ion-exchange resin. Following the final ion exchange, the filtrate was concentrated and recrystallized from acetonitrile and a small amount of ether to give 0.012 g (75%) of **AA1** as a dark blue solid; whose spectral properties were identical to those of an authentic sample,[9] mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.28 (s, 2 H), 7.97 (AA′BB′, 2 H, J = 9.5 Hz), 7.72 (AA′BB′, 4 H, J = 8.5 Hz), 6.88 (m, 6 H), 4.53 (s, 4 H), 3.21 (s, 6 H); λ_{max} (CH_2Cl_2) 581 nm, ϵ = 5.55 (± 0.05) × 10^4 M^{-1} cm^{-1}.

2,6-Bis(4-anilino)-4-(4-*N*,*N*-dimethylanilino)selenopyrylium Chloride (3-Se). A solution of Δ-4*H*-2,6-bis(4-anilino)-selenopyran-4-one (**5-Se**, 0.050 g, 0.15 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *p*-bromo-*N*,*N*-dimethylaniline, (0.29 g, 1.5 mmol) and Mg turnings, (0.07 g, 2.9 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for the preparation of **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.024 g (28%) of the PF_6 salt of **3-Se** as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.25 (s, 2 H), 7.98 (AA′BB′, 2 H, J = 9.5 Hz), 7.67 (AA′BB′, 4 H, J = 8.5 Hz), 6.87 (m, 6 H), 4.55 (s, 4 H), 3.19 (s, 6 H); λ_{max} (CH_2Cl_2) 610 nm, ϵ = 6.90 (± 0.02) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.020 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.014 g (86%) of the product as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.24 (s, 2 H), 7.97 (AA′BB′, 2 H, J = 9.5 Hz), 7.67 (AA′BB′, 4 H, J = 8.5 Hz), 6.88 (m, 6 H), 4.67 (s, 4 H), 3.18 (s, 6 H); λ_{max} (H_2O) 554 nm, ϵ = 3.23 (± 0.01) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2,6-Bis(4-anilino)-4-(4-*N*,*N*-dimethylanilino)telluropyrylium Chloride (3-Te). A solution of Δ-4*H*-2,6-(4-anilino)-telluropyran-4-one (**5-Te**, 0.050 g, 0.13 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *p*-bromo-*N*,*N*-dimethylaniline, (0.26 g, 1.3 mmol) and Mg turnings, (0.06 g, 2.6 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for the preparation of **AA1.** The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.017 g (21%) of the PF_6 salt of **3-Te** as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.29 (s, 2 H), 7.97 (AA′BB′, 2 H, J = 8.4 Hz), 7.58 (AA′BB′, 4 H, J = 8.5 Hz), 6.86 (m, 6 H), 4.56 (s, 4 H), 3.16 (s, 6 H); λ_{max} (CH_2Cl_2) 620 nm, ϵ = 3.96 (± 0.02) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.020 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.014 g (84%) of the product as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.26 (s, 2 H), 7.96 (AA′BB′, 2 H, J = 8.4 Hz), 7.57 (AA′BB′, 4 H, J = 8.5 Hz), 6.85 (m, 6 H), 4.73 (s, 4 H), 3.16 (s, 6 H); λ_{max} (H_2O) 581 nm, ϵ = 2.65 (± 0.08) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2,6-Bis(4-anilino)-4-(4-*N*-morpholinophenyl)thiopyrylium Chloride (4-S). A solution of Δ-4*H*-2,6-bis(4-anilino)-thiopyran-4-one (0.050 g, 0.17 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.41 g, 1.7 mmol) and Mg turnings, (0.08 g, 3.4 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.017 g (17%) of the product as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.29 (s, 2 H), 7.95 (AA′BB′, 2 H, J = 9.0 Hz), 7.75 (AA′BB′, 4 H, J = 8.5 Hz), 7.07 (AA′BB′, 2 H, J = 9.0 Hz), 6.87 (AA′BB′, 4 H, J = 8.5 Hz), 4.60 (s, 4 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.48 (t, 4 H, J = 4.8 Hz); λ_{max} (CH_2Cl_2) 566 nm, ϵ = 4.58 (± 0.02) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.020 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.012 g (74%) of **4-S** as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.28 (s, 2 H), 7.94 (AA′BB′, 2 H,

J = 9.0 Hz), 7.75 (AA′BB′, 4 H, J = 8.5 Hz), 7.07 (AA′BB′, 2 H, J = 9.0 Hz), 6.87 (AA′BB′, 4 H, J = 8.5 Hz), 4.60 (s, 4 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.48 (t, 4 H, J = 4.8 Hz); λ_{max} (H_2O) 535 nm, ϵ = 3.5 (± 0.1) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2,6-Bis(4-anilino)-4-(4-*N*-morpholinophenyl)selenopyrylium Chloride (4-Se). A solution of Δ-4*H*-2,6-bis(4-anilino)selenopyran-4-one (**5-Se**, 0.050 g, 0.15 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.36 g, 1.5 mmol) and Mg turnings, (0.08 g, 3.4 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.017 g (17%) of the product as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.25 (s, 2 H), 7.95 (AA′BB′, 2 H, J = 9.2 Hz), 7.70 (AA′BB′, 4 H, J = 8.8 Hz), 7.06 (AA′BB′, 2 H, J = 9.2 Hz), 6.86 (AA′BB′, 4 H, J = 8.8 Hz), 4.63 (s, 4 H), 3.87 (t, 4 H, J = 5.1 Hz), 3.46 (t, 4 H, J = 5.1 Hz); λ_{max} (CH_2Cl_2) 593 nm, ϵ = 4.48 (± 0.03) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.020 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.014 g (85%) of **4-Se** as a dark blue solid, mp >260 °C: ^{1}H NMR [400 MHz, CD_2Cl_2] δ 8.24 (s, 2 H), 7.93 (AA′BB′, 2 H, J = 9.2 Hz), 7.69 (AA′BB′, 4 H, J = 8.8 Hz), 7.06 (AA′BB′, 2 H, J = 9.2 Hz), 6.86 (AA′BB′, 4 H, J = 8.8 Hz), 4.70 (s, 4 H), 3.87 (t, 4 H, J = 5.1 Hz), 3.46 (t, 4 H, J = 5.1 Hz); λ_{max} (H_2O) 553 nm, ϵ = (3.15 ± 0.01) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2,6-Bis(4-anilino)-4-(4-*N*-morpholinophenyl)telluropyrylium Chloride (4-Te). A solution of Δ-4*H*-2,6-bis(4-anilino)telluropyran-4-one (**5-Te**, 0.050 g, 0.13 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.36 g, 1.5 mmol) and Mg turnings, (0.08 g, 3.4 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.017 g (17%) of the product as a dark blue solid, mp > 260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 2 H), 7.93 (AA′BB′, 2 H, J = 9.2 Hz), 7.61 (AA′BB′, 4 H, J = 8.9 Hz), 7.07 (AA′BB′, 2 H, J = 9.2 Hz), 6.84 (AA′BB′, 4 H, J = 8.9 Hz), 4.64 (s, 4 H), 3.87 (t, 4 H, J = 5.0 Hz), 3.42 (t, 4 H, J = 5.0 Hz); λ_{max} (CH_2Cl_2) 618 nm, ϵ = 5.38 (± 0.02) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.030 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.020 g (74%) of **4-Te** as a dark blue solid, mp >260 °C: ^{1}H NMR [400 MHz, CD_2Cl_2] δ 8.24 (s, 2 H), 7.90 (AA′BB′, 2 H, J = 9.2 Hz), 7.60 (AA′BB′, 4 H, J = 8.9 Hz), 7.06 (AA′BB′, 2 H, J = 9.2 Hz), 6.84 (AA′BB′, 4 H, J = 8.9 Hz), 4.74 (s, 4 H), 3.87 (t, 4 H, J = 5.0 Hz), 3.42 (t, 4 H, J = 5.0 Hz); λ_{max} (H_2O) 582 nm, ϵ = 2.28 (± 0.04) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-6-(4-*N*,*N*-dimethylanilino)-4-phenylselenopyrylium Chloride (11-Se). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*,*N*-dimethylanilino)selenopyran-4-one (**15-Se**, 0.074 g, 0.20 mmol) in THF (5 mL) was added dropwise to a 1.0-M solution of phenylmagnesium bromide in THF (1.0 mL, 1.0 mmol). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.027 g (15%) of the product as a dark blue solid, mp 180−183 °C: ^{1}H NMR [400 MHz, CD_2Cl_2] δ 8.24 (s, 1 H), 8.18 (s, 1 H), 7.96 (m, 2 H), 7.84 (AA′BB′, 2 H, J = 9.2 Hz), 7.76 (AA′BB′, 2 H, J = 8.8 Hz), 7.60 (m, 3 H), 7.04 (AA′BB′, 2 H, J = 9.2 Hz), 6.88 (AA′BB′, 2 H, J = 8.8 Hz), 4.93 (br s, 2 H), 3.18 (s, 6 H); λ_{max} (CH_2Cl_2) 670 nm, ϵ = 5.1 (± 0.2) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.030 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.020 g (75%) of **11-Se** as a dark blue solid, mp 187−188 °C: ^{1}H NMR [400 MHz, CD_2Cl_2] δ 8.24 (s, 1 H), 8.18 (s, 1 H), 7.96 (m, 2 H), 7.84 (AA′BB′, 2 H, J = 9.2 Hz), 7.76 (AA′BB′, 2 H, J = 8.8 Hz), 7.60 (m, 3 H), 7.04 (AA′BB′, 2 H, J = 9.2 Hz), 6.88 (AA′BB′, 2 H, J = 8.8 Hz), 4.93 (br s, 2 H), 3.18 (s, 6 H); λ_{max} (CH_2Cl_2) 635 nm, ϵ = 2.5 (± 0.1) × 10^4 M^{-1} cm^{-1}.

2-(4-Anilino)-6-(4-*N*-morpholinophenyl)-4-phenylthiopyrylium Chloride (12-S). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)thiopyran-4-one (**16-S**, 0.10 g, 0.27 mmol) in THF (5 mL) was added dropwise to a 1.0 M solution of phenylmagnesium bromide in THF (1.4 mL, 1.4 mmol). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.10 g (64%) of the hexafluorophosphate salt as a dark blue solid, mp 170−173 °C: ^{1}H NMR [400 MHz, CD_2Cl_2] δ 8.28 (s, 1 H), 8.27 (s, 1 H), 7.87 (m, 4 H), 7.82 (AA′BB′, 2 H, J = 8.8 Hz), 7.69 (m, 3 H), 7.06 (AA′BB′, 2 H, J = 9.2 Hz), 6.89 (AA′BB′, 2 H, J = 8.8 Hz), 4.83 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.48 (t, 4 H, J = 4.8 Hz); λ_{max} (CH_2Cl_2) 616 nm, ϵ = 4.40 (± 0.06) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.050 g) was dissolved in 20 mL of CH_3CN was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.040 g (98%) of **12-S** as a dark blue solid, mp 176−180 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.28 (s, 1 H), 8.27 (s, 1 H), 7.87 (m, 4 H), 7.82 (AA′BB′, 2 H, J = 8.8 Hz), 7.69 (m, 3 H), 7.06 (AA′BB′, 2 H, J = 9.2 Hz), 6.89 (AA′BB′, 2 H, J = 8.8 Hz), 4.83 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.48 (t, 4 H, J = 4.8 Hz); λ_{max} (H_2O) 597 nm, ϵ = 2.12 (± 0.09) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-6-(4-*N*-morpholinophenyl)-4-phenylselenopyrylium Chloride (12-Se). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)selenopyran-4-one (**16-Se**, 0.10 g, 0.24 mmol) in THF (5 mL) was added dropwise to a 1.0-M solution of phenylmagnesium bromide in THF (1.4 mL, 1.4 mmol). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.11 g (71%) of the hexafluorophosphate salt as a dark blue solid, mp 170−173 °C: ^{1}H NMR [400 MHz, CD_2Cl_2] δ 8.19 (s, 1 H), 8.18 (s, 1 H), 7.84 (m, 4 H), 7.76 (AA′BB′, 2 H, J = 8.8 Hz), 7.66 (m, 3 H), 7.04 (AA′BB′, 2 H, J = 9.2 Hz), 6.88 (AA′BB′, 2 H, J = 8.8 Hz), 4.93 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.47 (t, 4 H, J = 4.8 Hz); λ_{max} (CH_2Cl_2) 645 nm, ϵ = 4.9 (± 0.2) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.050 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.020 g (75%) of the product as a dark blue solid, mp 176−180 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.28 (s, 1 H), 8.27 (s, 1 H), 7.87 (m, 4 H), 7.82 (AA′BB′, 2 H, J = 8.8 Hz), 7.69 (m, 3 H), 7.06 (AA′BB′, 2 H, J = 9.2 Hz), 6.89 (AA′BB′, 2 H, J = 8.8 Hz), 4.83 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.48 (t, 4 H, J = 4.8 Hz); λ_{max} (H_2O) 597 nm, ϵ = 2.12 (± 0.09) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-6-(4-*N*-morpholinophenyl)-4-phenyltelluropyrylium Chloride (12-Te). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)telluropyran-4-one (**16-Te**, 0.10 g, 0.22 mmol) in THF (5 mL) was added dropwise to a 1.0-M solution of phenylmagnesium bromide in THF (1.1 mL, 1.1 mmol). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.090 g (63%) of the hexafluorophosphate salt as a dark blue solid, mp 190−193 °C: ^{1}H NMR [400 MHz, CD_2Cl_2] δ 8.17 (s, 1 H), 8.16 (s, 1 H), 7.81 (m, 2 H), 7.74 (AA′BB′, 2 H, J = 8.8 Hz), 7.65 (m, 5 H), 7.02 (AA′BB′, 2 H, J = 8.8 Hz), 6.86 (AA′BB′, 2 H, J = 8.4 Hz), 4.97 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.45 (t, 4 H, J = 4.8 Hz); λ_{max} (CH_2Cl_2) 674 nm, ϵ = 5.02 (± 0.07) × 10^2 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.050 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400

Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.020 g (75%) of **12-Te** as a dark blue solid, mp 176−180 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.15 (s, 1 H), 8.14 (s, 1 H), 7.81 (m, 2 H), 7.74 (AA′BB′, 2 H, J = 8.8 Hz), 7.65 (m, 5 H), 7.02 (AA′BB′, 2 H, J = 8.8 Hz), 6.86 (AA′BB′, 2 H, J = 8.4 Hz), 4.97 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.45 (t, 4 H, J = 4.8 Hz); λ_{max} (H_2O) 653 nm, $\epsilon = 256 \pm 9.5 \times 10^2$ M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-4,6-bis(4-*N*-morpholinophenyl)thiopyrylium Chloride (13-S). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)thiopyran-4-one (**16-S**, 0.05 g, 0.14 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.17 g, 0.69 mmol) and Mg turnings, (0.04 g, 1.7 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and ether to give 0.10 g (64%) of the hexafluorophosphate salt as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.31 (s, 1 H), 8.30 (s, 1 H), 7.96 (AA′BB′, 2 H, J = 8.5 Hz), 7.85 (AA′BB′, 2 H, J = 8.2 Hz), 7.76 (AA′BB′, 2 H, J = 8.2 Hz), 7.06 (m, 4 H), 6.88 (AA′BB′, 2 H, J = 8.5 Hz), 4.66 (s, 2 H), 3.86 (m, 8 H), 3.45 (m, 8 H); λ_{max} (CH_2Cl_2) 578 nm, $\epsilon = 6.9 (\pm 0.4) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.038 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.028 g (88%) of **13-S** as a dark blue solid, mp >260 °C:^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.29 (s, 1 H), 8.28 (s, 1 H), 7.96 (AA′BB′, 2 H, J = 8.5 Hz), 7.85 (AA′BB′, 2 H, J = 8.2 Hz), 7.76 (AA′BB′, 2 H, J = 8.2 Hz), 7.06 (m, 4 H), 6.88 (AA′BB′, 2 H, J = 8.5 Hz), 5.04 (s, 2 H), 3.86 (m, 8 H), 3.45 (m, 8 H); λ_{max} (H_2O) 546 nm, $\epsilon = 1.89 (\pm 0.05) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-4,6-bis(4-*N*-morpholinophenyl)selenopyrylium Chloride (13-Se). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)selenopyran-4-one (**16-Se**, 0.10 g, 0.24 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.29 g, 1.2 mmol) and Mg turnings, (0.060 g, 2.4 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and ether to give 0.13 g (77%) of the hexafluorophosphate salt as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 1 H), 8.26 (s, 1 H), 7.96 (AA′BB′, 2 H, J = 8.6 Hz), 7.79 (AA′BB′, 2 H, J = 8.2 Hz), 7.71 (AA′BB′, 2 H, J = 8.6 Hz), 7.07 (m, 4 H), 6.86 (AA′BB′, 2 H, J = 8.2 Hz), 4.67 (s, 2 H), 3.86 (m, 8 H), 3.43 (m, 8 H); λ_{max} (CH_2Cl_2) 608 nm, $\epsilon = 5.1 (\pm 0.1) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.049 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.039 g (95%) of **13-Se** as a dark blue solid, mp >260 °C:^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 1 H), 8.26 (s, 1 H), 7.96 (AA′BB′, 2 H, J = 8.6 Hz), 7.79 (AA′BB′, 2 H, J = 8.2 Hz), 7.71 (AA′BB′, 2 H, J = 8.6 Hz), 7.07 (m, 4 H), 6.86 (AA′BB′, 2 H, J = 8.2 Hz), 4.97 (s, 2 H), 3.86 (m, 8 H), 3.43 (m, 8 H); λ_{max} (H_2O) 566 nm, $\epsilon = 2.28 (\pm 0.07) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-4,6-bis(4-*N*-morpholinophenyl)telluropyrylium Chloride (13-Te). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)telluropyran-4-one (**16-Te**, 0.10 g, 0.22 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.26 g, 1.1 mmol) and Mg turnings, (0.060 g, 2.4 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and a small amount of ether to give 0.10 g (61%) of the hexafluorophosphate salt as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 1 H), 8.26 (s, 1 H), 7.93 (AA′BB′, 2 H, J = 9.2 Hz), 7.70 (AA′BB′, 2 H, J = 8.9 Hz), 7.61 (AA′BB′, 2 H, J = 8.5 Hz), 7.07 (AA′BB′, 2 H, J = 8.9 Hz), 7.02 (AA′BB′, 2 H, J = 9.2 Hz), 6.84 (AA′BB′, 2 H, J = 8.5 Hz), 4.67 (s, 2 H), 3.86 (m, 8 H), 3.42 (m, 8 H); λ_{max} (CH_2Cl_2) 638 nm, $\epsilon = 3.50 \pm 0.09) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.053 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN ether to give 0.037 g (82%) of **13-Te** as a dark blue solid, mp >260 °C:^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 1 H), 8.26 (s, 1 H), 7.93 (AA′BB′, 2 H, J = 9.2 Hz), 7.70 (AA′BB′, 2 H, J = 8.9 Hz), 7.61 (AA′BB′, 2 H, J = 8.5 Hz), 7.07 (AA′BB′, 2 H, J = 8.9 Hz), 7.02 (AA′BB′, 2 H, J = 9.2 Hz), 6.84 (AA′BB′, 2 H, J = 8.5 Hz), 4.84 (s, 2 H), 3.86 (m, 8 H), 3.42 (m, 8 H); λ_{max} (H_2O) 584 nm, $\epsilon = 2.38 (\pm 0.03) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-4-(4-*N*,*N*-dimethylanilino)-6-(4-*N*-morpholinophenyl)thiopyrylium Chloride (14-S). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)thiopyran-4-one (**16-S**, 0.10 g, 0.27 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from 4-bromo-*N*,*N*-dimethylaniline (0.28 g, 1.4 mmol) and Mg turnings, (0.07 g, 2.8 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and ether to give 0.12 g (72%) of the hexafluorophosphate salt as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.30 (s, 1 H), 8.27 (s, 1 H), 7.98 (AA′BB′, 2 H, J = 9.2 Hz), 7.82 (AA′BB′, 2 H, J = 8.9 Hz), 7.73 (AA′BB′, 2 H, J = 9.2 Hz), 7.05 (AA′BB′, 2 H, J = 9.2 Hz), 7.02 (AA′BB′, 2 H, J = 9.2 Hz), 6.86 (AA′BB′, 2 H, J = 8.9 Hz), 4.55 (s, 2 H), 3.86 (t, 4 H, J = 4.9 Hz), 3.40 (t, 4 H, J = 4.9 Hz), 3.20 (s, 6 H); λ_{max} (CH_2Cl_2) 589 nm, $\epsilon = 7.7 (\pm 0.2) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.050 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and ether to give 0.039 g (95%) of **14-S** as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.30 (s, 1 H), 8.29 (s, 1 H), 7.98 (AA′BB′, 2 H, J = 9.2 Hz), 7.83 (AA′BB′, 2 H, J = 9.2 Hz), 7.73 (AA′BB′, 2 H, J = 8.9 Hz), 7.05 (AA′BB′, 2 H, J = 9.2 Hz), 7.02 (AA′BB′, 2 H, J = 9.2 Hz), 6.86 (m, 4 H), 4.64 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.40 (t, 4 H, J = 4.8 Hz), 3.20 (s, 6 H); λ_{max} (H_2O) 553 nm, $\epsilon = 1.55 (\pm 0.09) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-4-(4-*N*,*N*-dimethylanilino)-6-(4-*N*-morpholinophenyl)selenopyrylium Chloride (14-Se). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)selenopyran-4-one (**16-Se**, 0.10 g, 0.24 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from 4-bromo-*N*,*N*-dimethylaniline (0.28 g, 1.4 mmol) and Mg turnings (0.07 g, 2.8 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and ether to give 0.12 g (72%) of the hexafluorophosphate salt as a dark blue solid, mp 179−183 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 1 H), 8.26 (s, 1 H), 7.99 (AA′BB′, 2 H, J = 9.2 Hz), 7.77 (AA′BB′, 2 H, J = 9.1 Hz), 7.68 (AA′BB′, 2 H, J = 8.5 Hz), 7.04 (AA′BB′, 2 H, J = 9.2 Hz), 6.95 (AA′BB′, 2 H, J = 9.1 Hz), 6.87 (AA′BB′, 2 H, J = 8.5 Hz), 4.59 (s, 2 H), 3.86 (t, 4 H, J = 4.6 Hz), 3.40 (t, 4 H, J = 4.6 Hz), 3.19 (s, 6 H); λ_{max} (CH_2Cl_2) 616 nm, $\epsilon = 5.3 (\pm 0.2) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.047 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.037 g (95%) of **14-Se** as a dark blue solid, mp 187−190 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 1 H), 8.26 (s, 1 H), 7.97 (AA′BB′, 2 H, J = 9.5 Hz), 7.77 (AA′BB′, 2 H, J = 8.9 Hz), 7.68 (AA′BB′, 2 H, J = 8.9 Hz), 7.04 (AA′BB′, 2 H, J = 8.9 Hz), 6.90 (AA′BB′, 2 H, J = 9.5 Hz), 6.86 (AA′BB′, 2 H, J = 8.5 Hz), 4.63 (s, 2 H), 3.86 (t, 4 H, J = 4.6 Hz), 3.40 (t, 4 H, J = 4.6 Hz), 3.19 (s, 6 H); λ_{max} (H_2O) 557 nm, $\epsilon = 3.32 (\pm 0.06) \times 10^4$ M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-4-(4-*N*,*N*-dimethylanilino)-6-(4-*N*-morpholinophenyl)telluropyrylium Chloride (14-Te). A solution of Δ-4*H*-2-(4-anilino)-6-(4-*N*-morpholinophenyl)selenopyran-4-one (**16-Te**, 0.10 g, 0.22 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from 4-bromo-*N*,*N*-dimethylaniline (0.28 g, 1.4 mmol) and Mg turnings, (0.07 g, 2.8 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The product was recrystallized from CH_3CN and ether to give 0.13 g (85%) of the hexafluorophosphate salt as a blue solid, mp 180−184 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.30 (s, 1 H), 8.28 (s, 1 H), 7.98 (AA′BB′, 2 H, J = 9.2 Hz), 7.68 (AA′BB′, 2 H, J = 8.9 Hz), 7.59 (AA′BB′, 2 H, J = 8.9 Hz), 7.02 (AA′BB′, 2 H, J = 8.5 Hz), 6.95 (AA′BB′, 2 H, J = 9.2 Hz), 6.84 (AA′BB′, 2 H, J = 8.5 Hz), 4.58 (s, 2 H), 3.86 (t, 4 H, J = 4.8 Hz), 3.39 (t, 4 H, J = 4.8 Hz), 3.16 (s, 6 H); λ_{max} (CH_2Cl_2) 646 nm, ϵ = 7.3 (± 0.1) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.045 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and ether to give 0.037 g (95%) of **14-Te** as a dark blue solid, mp 187−190 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.28 (s, 1 H), 8.27 (s, 1 H), 7.96 (AA′BB′, 2 H, J = 9.2 Hz), 7.67 (AA′BB′, 2 H, J = 9.2 Hz), 7.58 (AA′BB′, 2 H, J = 8.5 Hz), 7.01 (AA′BB′, 2 H, J = 9.2 Hz), 6.90 (AA′BB′, 2 H, J = 9.2 Hz), 6.83 (AA′BB′, 2 H, J = 8.5 Hz), 4.70 (s, 2 H), 3.85 (t, 4 H, J = 4.8 Hz), 3.39 (t, 4 H, J = 4.8 Hz), 3.15 (s, 6 H); λ_{max} (H_2O) 583 nm, ϵ = 3.56 (± 0.08) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2-(4-Anilino)-4-(4-*N*-morpholinophenyl)-6-phenylselenopyrylium Chloride (21-Se). A solution of Δ-4*H*-2-(4-anilino)-6-phenylselenopyran-4-one[8c] (0.050 g, 0.15 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.37 g, 1.5 mmol) and Mg turnings, (0.07 g, 2.8 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 0.5 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and ether to give 0.080 g (90%) of the hexafluorophosphate salt as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.44 (s, 1 H), 8.38 (s, 1 H), 8.04 (AA′BB′, 2 H, J = 9.2 Hz), 7.73 (m, 7 H), 7.08 (AA′BB′, 2 H, J = 9.2 Hz), 6.89 (AA′BB′, 2 H, J = 8.9 Hz), 6.87 (AA′BB′, 2 H, J = 8.5 Hz), 4.81 (s, 2 H), 3.87 (t, 4 H, J = 5.0 Hz), 3.51 (t, 4 H, J = 5.0 Hz); λ_{max} (CH_2Cl_2) 611 nm, ϵ = 5.5 (± 0.2) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.050 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and ether to give 0.031 g (76%) of **21-Se** as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.39 (s, 1 H), 8.32 (s, 1 H), 8.00 (AA′BB′, 2 H, J = 9.5 Hz), 7.76 (m, 7 H), 7.07 (AA′BB′, 2 H, J = 9.2 Hz), 6.89 (AA′BB′, 2 H, J = 8.9 Hz), 4.81 (s, 2 H), 3.86 (t, 4 H, J = 4.6 Hz), 3.49 (t, 4 H, J = 4.6 Hz); λ_{max} (H_2O) 580 nm, ϵ = 2.41 (±0.05) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

2,4,6-Tris(4-*N*-morpholinophenyl)selenopyrylium Chloride (27-Se). A solution of Δ-4*H*-2,6-Bis(4-*N*-morpholinophenyl)selenopyran-4-one (**26-Se**, 0.10 g, 0.21 mmol) in THF (5 mL) was added dropwise to the Grignard reagent prepared from *N*-(4-bromophenyl)morpholine (0.25 g, 1.0 mmol) and Mg turnings, (0.05 g, 2.1 mmol) in THF (2 mL). The resulting mixture was heated at reflux for 2 h and was treated as described for **AA1**. The crude product was recrystallized from CH_3CN and ether to give 0.080 g (90%) of the hexafluorophosphate salt as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.27 (s, 2 H), 7.97 (AA′BB′, 2 H, J = 9.2 Hz), 7.81 (AA′BB′, 4 H, J = 9.2 Hz), 7.06 (m, 6 H), 3.86 (t, 12 H, J = 4.8 Hz), 3.45 (m, 12 H); λ_{max} (CH_2Cl_2) = 622 nm, ϵ = 6.71 (± 0.09) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

The hexafluorophosphate salt (0.049 g) was dissolved in 20 mL of CH_3CN and was treated with Amberlite IRA-400 Chloride ion-exchange resin as described for **AA1**. The product was recrystallized from CH_3CN and a small amount of ether to give 0.040 g (95%) of **21-Se** as a dark blue solid, mp >260 °C: ^{1}H NMR [500 MHz, CD_2Cl_2] δ 8.30 (s, 2 H), 8.02 (AA′BB′, 2 H, J = 9.2 Hz), 7.84 (AA′BB′, 4 H, J = 9.2 Hz), 7.07 (m, 6 H), 3.86 (t, 12 H, J = 4.8 Hz), 3.45 (m, 12 H); λ_{max} (H_2O) = 582 nm, ϵ = 1.78 (± 0.02) × 10^4 M^{-1} cm^{-1}. Anal. C, H, N.

Electrochemical Procedures. A BAS 100 potentiostat/galvanostat and programmer were used for the electrochemical measurements. The working electrode for cyclic voltammetry was a platinum disk electrode (diameter, 1 mm) obtained from Princeton Applied Research. The auxiliary and reference electrodes were silver wires. The reference for cyclic voltammetry was the Fc/Fc^+ couple at +0.40 V at a scan rate of 0.1 V s^{-1}. All samples were run in J. T. Baker HPLC-grade dichloromethane that had been stored over 3A molecular sieves and freshly distilled prior to use. Tetrabutylammonium fluoroborate (Aldrich Chemical Co.) was recrystallized from EtOAc−ether and then dried overnight at 80 °C before it was used as supporting electrolyte at 0.2 M. Nitrogen was used for sample deaeration.

Determination of Partition Coefficients. The octanol/water partition coefficients were all measured at pH 6 (phosphate-buffered) using UV−visible spectrophotometry. The measurements were done using a "shake flask" direct measurement.[20] Mixing for 3−5 min was followed by 1 h of settling time. Equilibration and measurements were made at 23 °C using a Perkin-Elmer Lambda 12 spectrophotometer. HPLC grade 1-octanol was obtained from Sigma-Aldrich.

Cells and Culture Conditions. Colo-26, a murine colon carcinoma cell line, was maintained in a growth medium of RPMI 1640 supplemented with 10% fetal calf serum (FCS) 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 a growth medium of Minimum Essential Media (α-MEM) supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals, Atlanta, GA), 50 units/mL of penicillin G, 50 mg/mL of streptomycin, and 1.0 mg/mL of Fungizone.

In Vitro Phototoxicity Measurements. Colo-26 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 phosphate-buffered saline (PBS), and 100 μL of Hank's basic salt solution (HBSS) with 1% FCS containing various concentrations of dyes **AA1**, **1**−**4**, **11**−**14**, **21-Se**, or **27-Se** was added to each well. The dye and cells were incubated for 2 h at 37 °C followed by a wash with PBS and the addition of 100 μL of PBS supplemented with 1% FCS. The plates were irradiated with filtered 360-to-800-nm light for a total light dose of 15 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. Control cells (no dye + light; dye + no light) were treated using the same conditions. Cell survival was monitored using the MTT assay as described in Mosmann.[24]

Cytochrome *c* Oxidase Measurements in Cultured R3230AC Tumor Cells. Cells cultured from the rodent mammary adenocarcinomas (R3230AC) were used for these studies. The R3230AC tumors were maintained by transplantation in the abdominal region of 100−120 g Fischer female rats, using the sterile trochar technique.[25] R3230AC cells were cultured from tumor homogenates using the method described earlier.[26] Cells were maintained in passage culture on 100-mm diameter polystyrene dishes (Becton Dickinson, Franklin Lakes, NJ) in 10 mL of α-MEM supplemented with 10% FBS, 50 units/mL penicillin G, 50 ug/mL streptomycin, and 1.0ug/mL Fungizone. Only cells from passages 1−10 were used for experiments. A stock of cells, passages 1−4, were maintained at −86 °C to initiate the experimental cultures. Cultures were maintained at 37 °C in a 5% CO_2 humidified atmosphere (Forma Scientific, Marietta, OH). Passage was accomplished by removing the culture medium, then adding a 1 mL solution containing 0.25% trypsin, incubating at 37 °C for 2 to 5 min until cells were detached from the surface followed by seeding new culture dishes with an appropriate number of cells in 10 mL of α-MEM. Cell counts were performed using a particle counter (Model ZM, Coulter Electronics, Hialeah, FL).

To determine whether **2-Se** photosensitized mitochondrial cytochrome *c* oxidase activity, R3230AC cells were plated on 12 well culture plates using an initial cell seeding of 1.0×10^5 cells/well in 1.0 mL of α-MEM with 10% FBS. Cells were incubated for 24 to 48 h at 37 °C in a 5% CO_2 humidified atmosphere until they reached 4 to 7×10^5 cells/well, a number where the cells were still in log phase growth. The α-MEM was removed and fresh α-MEM with 10% FBS, with dye at 1×10^{-8}, 1×10^{-7}, and 1×10^{-6} final concentration, was added. Control cells, with no added dye, were maintained under the same conditions. Cells were incubated for 24 h in the dark, the medium was removed, the cells were washed once with α-MEM minus FBS and phenol (no dye in the wash), and 1.0 mL of fresh α-MEM minus phenol but supplemented with 10% FBS was added. Monolayers on culture plates with lids removed were then exposed to 360-to-750-nm light from a tungsten−halogen source (0.5 mW/cm^2) at ambient temperature. At the end of the irradiation period (10, 20, or 30 min), the medium was removed and replaced with 1.0 mL of fresh α-MEM containing phenol and 10% FBS and incubated for 24 h. The media was removed, 0.1 mL of trypsin was added to each well, and cells were incubated at 37 °C for 3−5 min until all the cells detached from the surface. Cell suspensions were transferred to 1.0-mL microcentrifuge tubes and centrifuged at 8000*g* for 3 min. The supernatant was aspirated, and cell pellets were immediately frozen and stored at −86 °C. Cytochrome *c* oxidase activity was determined on cells that had been thawed and sonicated for 10 s using a Bronson sonicator (Model 185, Brinkmann Ind.) at a setting of 2. A sonicated cell suspension, representing 1 to 1.5×10^6 cells, was used for measurements of cytochrome *c* oxidase according to the method described earlier.[21] Data are expressed as percent of control cytochrome *c* oxidase activity, moles of cytochrome *c* oxidized/min/cell, which was determined from cells not exposed to dye or light. Cytochrome *c* oxidase activity was also determined in cells exposed to dye alone or light alone, drug, and light controls, respectively.

For dyes **1-S**, **3-Se**, **4-Se**, **13-Te**, and **14-Se,** cells were incubated for 24 h in the dark as above, with 1.0×10^{-6} M dye. Cultures were washed once with 0.9% NaCl and irradiated for 1 h with 360-to-750-nm light from a filtered tungsten−halogen source delivered at 0.5 mW/cm^2 (1.8 J cm^{-2}). Immediately following irradiation, the saline was removed from the substrate and replaced with media (MEM + 10% FBS). Both irradiated cells and dye-treated dark controls were incubated for 24 h in the dark and the activity of cytochrome *c* oxidase was determined as described above.

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.

Acknowledgment. The authors thank the National Institutes of Health (Grant CA69155 to M.R.D., Grant CA55791 to A. R. O., and Grant CA36856 to R.H.) and the shared resources of the Roswell Park Cancer Center support grant (P30 CA16056) for partial support of this work.

Supporting Information Available: Figure S1 showing LD_{50} as a function of *E*°; Figure S2 showing LD_{50} as a function of log *P*; Figure S3 showing LD_{50}/EC_{50} as a function of *E*°; experimental procedures for the preparation of compounds **5**, **6**, *N*-(4-iodophenyl)morpholine, **15**, **16**, **18**−**20**, and **23**−**26**; and plots of dark and phototoxicity for dyes **2-Se**, **3-Se**, **4-Se**, **13**, **14-Se**, and **27-Se**. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Sharman, W. M. Allen, C. M.; van Lier, J. E. Photodynamic Therapeutics: Basic Principles and Clinical Applications. *Drug Discovery Today* **1999**, *4*, 507−517. (b) Detty, M. R. Photosensitisers for the Photodynamic Therapy of Cancer and Other Diseases. *Expert Opin. Ther. Patents* **2001**, *11*, 1849−1860.

(2) (a) Krämer, S. D. Absorption Prediction from Physicochemical Parameters. *Pharm. Sci. Technol. Today* **1999**, *2*, 373−380. (b) Beresford, A. P.; Selick, H. E.; Tarbit, M. H. The Emerging Importance of Predictive ADME Simulation in Drug Discovery. *Drug Discovery Today* **2002**, *7*, 109−116.

(3) (a) Davis, S.; Weiss, M. J.; Wong, J. R.; Lampidis, T. J.; Chen, L. B. Mitochondrial and Plasma Membrane Potentials Cause Unusual Accumulation and Retention of Rhodamine 123 by Breast Adenocarcinoma-Derived MCF-7 Cells. *J. Biol. Chem.* **1985**, *260*, 13844−13850. (b) Lampidis, T. J.; Bernal, S. D.; Summerhayes, I. C.; Chen, L. B. Selective Toxicity of Rhodamine-123 in Carcinoma Cells in Vitro. *Cancer Res.* **1983**, *43*, 716−720. (c) Bernal, S. D.; Lampidis, T. J.; McIsaac, R. M.; Chen, L. B. Anticarcinoma Activity in vivo of Rhodamine 123, a Mitochondrial-Specific Dye. *Science* **1986**, *222*, 169−172. (d) Modica-Napolitano, J. S.; Aprille, J. R. Basis for the Selective Cytotoxicity of Rh123. *Cancer Res.* **1987**, *47*, 4361−4365. (e) Nadakavukaren, K. K.; Nadakavukaren, J. J.; Chen, L. B. Increased Rhodamine 123 Uptake by Carcinoma Cells. *Cancer Res.* **1986**, *45*, 6093−6099. (f) Wong, J. R.; Chen, L. B. Recent Advances in the Study of Mitochondria in Living Cells. *Adv. Cell Biol.* **1988**, *2*, 263−290. (g) Modica-Napolitano, J. S.; Joyal, J. L.; Ara, G.; Oseroff, A. R.; Aprille, J. R. Mitochondrial Toxicity of Cationic Photosensitizers for Photochemotherapy. *Cancer Res.* **1990**, *50*, 7876−7881. (h) Morgan, J.; Whitaker, J. E.; Oseroff, A. R. GRP78 Induction by Calcium Ionophore Potentiates Photodynamic Therapy Using the Mitochondrial Targeting Dye Victoria Blue BO. *Photochem. Photobiol.* **1998**, *67*, 155−164. (i) Noodt, B. B.; Rodal, G. H.; Wainwright, M.; Peng, Q.; Horobin, R.; Nesland, J. M.; Berg, K. Apoptosis Induction by Different Pathways with Methylene Blue Derivative and Light from Mitochondrial Sites in V79 Cells. *Int. J. Cancer* **1998**, *75*, 941−948.

(4) (a) Cincotta, L.; Foley, J. W.; Cincotta, A. H. Phototoxicity, Redox Behavior, and Pharmacokinetics of Benzophenoxazine Analogues in EMT-6 Murine Sarcoma Cells. *Cancer Res.* **1993**, *53*, 2571−2580. (b) Lin, C. W.; Shulok, J. R.; Kirley, S. D.; Bachelder, C. M.; Flotte, T. J.; Sherwood, M. E.; Cincotta, L.; Foley, J. W. Photodynamic Destruction of Lysosomes Mediated by Nile Blue Photosensitizers. *Photochem. Photobiol.* **1993**, *58*, 81−91. (c) Lin, C. W.; Shulok, J. R.; Kirley, S. D.; Cincotta, L.; Foley, J. W. Lysosomal Localization and Mechanism of Uptake of Nile Blue Photosensitizers in Tumor Cells. *Cancer Res.* **1991**, *51*, 2710−2719. (d) Cincotta, L.; Foley, J. W.; MacEachern, T.; Lampros, E.; Cincotta, A. H. Novel Photodynamic Effects of a Benzophenothiazine on Two Different Murine Sarcomas. *Cancer Res.* **1994**, *54*, 1249−1258.

(5) (a) Oseroff, A. R.; Ohuoha, D.; Ara, G.; McAuliffe, D.; Foley, J.; Cincotta, L. Intramitochondrial Dyes Allow Selective in vitro Photolysis of Carcinoma. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 9729−9733. (b) Ara, G.; Aprille, J. R.; Malis, C. D.; Kane, S. B.; Cincotta, L.; Foley, J.; Bonventre, J. V.; Oseroff, A. R. Mechanisms of Mitochondrial Photosensitization by the Cationic Dye, *N,N′*-Bis(2-ethyl-1,3-dioxylene)kryptocyanine (EDKC): Preferential Inactivation of Complex I in the Electron Transport Chain. *Cancer Res.* **1987**, *47*, 6580−6585. (c) Lee, C.; Wu, S. S.; Chen, L. B. Photosensitization by 3,3′-Dicyclohexyloxacarbocyanine Iodide: Specific Disruption of Microtubules and Inactivation of Organelle Motility. *Cancer Res.* **1995**, *55*, 2063−2069.

(6) (a) Modica-Napolitano, J. S.; Brunelli, B. T.; Koya, K.; Chen, L. B. Photoactivation Enhances the Mitochondrial Toxicity of the Cationic Rhodacyanine MKT-077. *Cancer Res.* **1998**, *58*, 71−75. (b) Modica-Nopolitano, J. S.; Koya, K.; Weisberg, E.; Brunelli, B. T.; Li, Y.; Chen, L. B. Selective Damage to Carcinoma Mitochondria by the Rhodacyanine MKT-077. *Cancer Res.* **1996**, *56*, 544−550.

(7) (a) Detty, M. R.; Merkel, P. B.; Hilf, R.; Gibson, S. L.; Powers, S. K. Chalcogenapyrylium Dyes as Photochemotherapeutic Agents. 2. Tumor Uptake, Mitochondrial Targeting, and Singlet-Oxygen-Induced Inhibition of Cytochrome *c* Oxidase. *J. Med. Chem.* **1990**, *33*, 1108−1116. (b) Kessel, D. Intracellular Localization of a Chalcogenapyrylium Dye Probed by Spectroscopy and Sites of Photodamage. *Photochem. Photobiol.* **1991**, *53*, 73−78. (c) Powers, S. K.; Walstad, D. L.; Brown, J. T.; Detty, M. R.; Watkins, P. J. Photosensitization of Human Glioma Cells by Chalcogenapyrylium Dye. *J. Neuro-Oncol.* **1989**, *7*, 179−187.

(8) (a) Leonard, K. A.; Nelen, M. I.; Anderson, L. T.; Gibson, Scott 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. (b) Leonard, K. A.; Nelen, M. I.; Simard, T. P.; Davies, S. R.; Gollnick, S. O.; Oseroff, A. R.; Gibson, S. L.; Hilf, R.; Chen, L. B.; Detty, M. R. Synthesis and Evaluation of Chalcogenopyrylium Dyes as Potential Sensitizers for the Photodynamic Therapy of Cancer. *J. Med. Chem.* **1999**, *42*, 3953−3964. (c) Leonard, K. A.; Hall, J. P.; Nelen, M. I.; Davies, S. R.; Gollnick, S. O.; Camacho, S.; Oseroff, A. R.; Gibson,

S. L.; Hilf, R.; Detty, M. R. A Selenopyrylium Photosensitizer for Photodynamic Therapy Related in Structure to the Antitumor Agent AA1 with Potent in Vivo Activity and No Long-term Skin Photosensitization. *J. Med. Chem.* **2000**, *43*, 4488−4498.

(9) Sun, X.; Wong, J. R.; Song, K.; Hu, J.; Garlid, K. D.; Chen, L. B. AA1, A Newly Synthesized Monovalent Lipophilic Cation, Expresses Potent in vivo Antitumor Activity. *Cancer Res.* **1994**, *54*, 1465−1471.

(10) Millish, K. J.; Cox, R. D.; Vernon, D. I.; Griffiths, J.; Brown, S. B. In Vitro Photodynamic Activity of a Series of Methylene Blue Analogues. *Photochem. Photobiol.* **2002**, *75*, 392−397.

(11) Melissaris, A. P.; Litt, M. H. A Simple and Economical Synthetic Route to *p*-Ethynylaniline and Ethynyl-Terminated Substrates. *J. Org. Chem.* **1994**, *59*, 5818−5821.

(12) Djuric, S.; Venit, J.; Magnus, P. Silicon in Synthesis: Stabase Adducts − A New Primary Amine Protecting Group: Alkylation of Ethyl Glycinate. *Tetrahedron Lett.* **1981**, *22*, 1787−1790.

(13) (a) Detty, M. R.; Murray, B. J. Telluropyrylium Dyes. 1. 2,6-Diphenyltelluropyrylium Dyes. *J. Org. Chem.* **1982**, *47*, 5235−5242. (b) Leonard, K.; Nelen, M.; Raghu, M.; Detty, M. R. Chalcogenopyranones from Disodium Chalcogenide Additions to 1,4-Pentadiyn-3-ones. The Role of Enol Ethers as Intermediates. *J. Heterocycl. Chem.* **1999**, *36*, 707−717.

(14) Majetich, G.; Hicks, R.; Reister, S. Electrophilic Aromatic Bromination Using Bromodimethylsulfonium Bromide Generated in Situ. *J. Org. Chem.* **1997**, *62*, 4321−4326.

(15) La Clair, J. J. Selective Detection of the Carbohydrate-Bound State of Concanvalin A at the Single Molecule Level. *J. Am. Chem. Soc.* **1997**, *119*, 7676−7684.

(16) Effenberger, F.; Agster, W.; Fischer, P.; Jogun, K. H.; Stezowski, J. J.; Daltrozzo, E.; Kolmannsberger-von Nell, G. Synthesis, Structure and Spectral Behavior of Donor−Acceptor Substituted Biphenyls. *J. Org. Chem.* **1983**, *48*, 4649−4658.

(17) Terpugova, M. P.; Kotlyarevskii, I. L.; Amosov, Y. I.; Myasnikova, R. N. Synthesis of *N*,*N*-Dialkylaminophenylacetylenes. *Bull. Acad. Sci. USSR Div. Chem. Sci. EN*, **1975**, *24*, 2697−99.

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

(19) (a) Young, D. N.; Detty, M. R. Hydrolysis Studies of Chalcogenopyrylium Trimethine Dyes. I. Product Studies in Alkaline Solution (pH ≥ 8) under Anaerobic and Aerobic Conditions. *J. Org. Chem.* **1997**, *62*, 4692−4700. (b) Young, D. N.; Serguievski, P.; Detty, M. R. Hydrolysis Studies of Chalcogenopyrylium Trimethine Dyes. 2. Chalcogen Atom Effects on the Rates of Hydrolysis of Chalcogenopyrylium Dyes. *J. Org. Chem.* **1998**, *63*, 5716−5721. (c) Bellnier, D. A.; Young, D. N.; Detty, M. R.; Camacho, S.; Oseroff, A. R. pH-Dependent Chalcogenopyrylium Dyes as Potential Sensitizers for PDT. Selective Retention in Tumor by Exploiting pH Differences between Tumor and Normal Tissue. *Photochem. Photobiol.* **1999**, *70*, 630−636.

(20) Sangster, J. In *Octanol−Water Partition Coefficients: Fundamentals and Physical Chemistry*; Wiley Series in Solution Chemistry; Fogg, P. G. T., Ed.; John Wiley and Sons: New York, 1997.

(21) Gibson, S. L.; Hilf, R. Photosensitization of Mitochondrial Cytochrome *c* Oxidase by Hematoporphyrin Derivative and Related Porphyrins in Vitro and in Vivo. *Cancer Res.* **1983**, *43*, 4191−4197.

(22) Lin, C.-W.; Shulok, J. R.; Kirley, S. D.; Cincotta, L.; Foley, J. W. Lysosomal Localization and Mechanism of Uptake of Nile blue Photosensitizers in Tumor Cells. *Cancer Res.* **1991**, *51*, 2710−2719.

(23) (a) Woodburn, K. W.; Vardaxis, N. J.; Hill, J. S.; Kaye, A. H.; Reiss, J. A.; Phillips, D. R. Evaluation of Porphyrin Characteristics Required for Photodynamic Therapy. *Photochem. Photobiol.* **1992**, *55*, 697−704. (b) Berg, K.; Madslien, K.; Bommer, J. C.; Ofteboro, R.; Winkelman, J. W.; Moan, J. Light Induced Relocalization of Sulfonated *meso*-Tetraphenylporphyrines in NHIK 3025 Cells and Effects of Dose Fractionation. *Photochem. Photobiol.* **1991**, *53*, 203-210.

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

(25) Hilf, R.; Michel, I.; Bell, C.; Freeman, J. J.; Borman, A. Biochemical and Morphological Properties of a New Lactating Tumor Line in the Rat. *Cancer Res.* **1965**, *25*, 286−299.

(26) Hissin, P. J.; Hilf, R. Effects of Insulin in Vivo and in Vitro on Amino Acid Transport into Cells from R3230AC Mammary Adenocarcinoma and Their Relationship to Tumor Growth. *Cancer Res.* **1978**, *38*, 3646−3651.

JM020260M