

Heteroatom Substitution Induced Changes in Excited-State Photophysics and Singlet Oxygen Generation in Chalcogenoxanthylum Dyes: Effect of Sulfur and Selenium Substitutions[†]

Tymish Y. Ohulchanskyy, David J. Donnelly, Michael R. Detty, and Paras N. Prasad*

Institute for Lasers, Photonics and Biophotonics, Department of Chemistry, State University of New York, Buffalo, New York

Received: October 10, 2003; In Final Form: March 31, 2004

Photophysical properties of a series of tetramethylrosamine dyes in which the xanthylum oxygen atom has been replaced with sulfur or selenium were examined. Dyes with sulfur and, especially, with selenium show decreased fluorescence quantum yield and lifetimes, while at the same time proportionally larger increases in the rates of intersystem crossing lead to higher triplet yields (0.23 and 0.97, correspondingly). The selenium-containing dye (TMR–Se) displays prominent phosphorescence at 77 K and at room temperature when the solution is purged of oxygen. This is reflected in a high yield for singlet oxygen generation as shown by measurements of singlet oxygen phosphorescence. The high singlet oxygen yield for TMR–Se (0.87) suggests its use as an efficient photosensitizer for the generation of singlet oxygen in biology and chemistry applications.

Introduction

Cationic dyes based on the xanthylum nucleus are important chromophores in chemistry and biology. As examples of photochemical applications, the rhodamine and rosamine dyes have been used as laser dyes, fluorescent labels, and fluorescence emission standards where their high fluorescence quantum yield and photostability are exploited.^{1–5} Because of the low triplet yield associated with rhodamine and rosamine dyes (typically, 1% or less),⁶ most photophysical studies of these materials concentrate on singlet-state properties. However, dye photostability is, to a great extent, determined by the metastable triplet state, which makes the study of the rhodamine and rosamine triplet states of interest.

Rhodamine and rosamine dyes are useful fluorescence probes in cell biology studies, showing specific fluorescence staining of mitochondria and other cell organelles.⁷ Irradiation of the rhodamines in biological studies reveals some phototoxicity⁸ and suggests their possible use as photosensitizers for the photodynamic therapy (PDT) of cancer.^{9,10} In this approach, the ability of the xanthylum dye to produce singlet oxygen (¹O₂), which is one of the main cytotoxic agents in PDT,¹¹ is of importance. Photosensitization of singlet oxygen is considered to occur mainly through the triplet state of the photosensitizer.^{12,13} Rhodamine analogues with increased triplet yields have generated significant interest.¹⁴ The tetrabromo rhodamine derivatives illustrate the well-known heavy-atom effect, which consists of an increase in spin–orbit coupling with substitution of atoms of higher atomic number and, thereby, an increase in the probability of intersystem crossing, $S_1 \rightarrow T_1$.¹² Consistent with their improved photophysical properties, brominated rhodamines have increased phototoxicity in vitro and target the mitochondria like their light-atom counterparts.^{10,15} The reported triplet yields (0.27–0.38) as well as the singlet oxygen yields (0.30–0.50, depending upon the solvent) are not exceptionally

high, but they are significantly larger than those of unmodified rhodamines.¹⁶

In earlier studies, the substitution of heavier chalcogen atoms for oxygen in a series of chalcogenopyrylium dyes gave longer wavelengths of absorption and increased triplet yields and singlet oxygen yields.¹⁷ Even though radiative lifetimes decreased with increasing chalcogen size, rates of intersystem crossing increased dramatically with increasing chalcogen size, leading to the observed effects. In the present paper, we have examined the photophysical properties of a series of tetramethylrosamine dyes in which the xanthylum oxygen atom has been replaced with sulfur or selenium (Figure 1).

Xanthylum dyes with high triplet yields from internal heavy-atom effects (eosinY, rose bengal, erythrosine B, phloxine B) are interesting molecules for study because their triplet states can be easily populated using excitation by a convenient laser source. Moreover, heavy-atom effects also make possible back-intersystem crossing that can be used to populate singlet levels through triplet ones.^{18–20} The series of tetramethylrosamine dyes in Figure 1 is of interest not only for the potential application to PDT and biological labels but also for the examination of the effects of heavy atoms on excited states and photophysical properties.

Experimental Section

Materials. TMR–O, TMR–S, and TMR–Se were prepared from the corresponding chalcogenoxanthone according to literature methods.²¹

Rhodamine 6G and Rose Bengal were purchased from Fisher Scientific (Hampton, NH) and were used as the references during fluorescence and singlet oxygen yields measurements. Methanol was purchased from J. T. Baker (Phillipsburg, NJ) and was used for solution preparation without purification.

Instrumentation and Methods. Steady-state measurements at room temperature were performed using a Shimadzu UV-3101PC spectrophotometer (absorption spectra) and Fluorolog-3 spectrofluorometer (Jobin Yvon) (fluorescence spectra). Fluor-

[†] Part of the special issue “Alvin L. Kwiram Festschrift”.

* To whom correspondence may be addressed. E-mail: pnprasad@buffalo.edu.

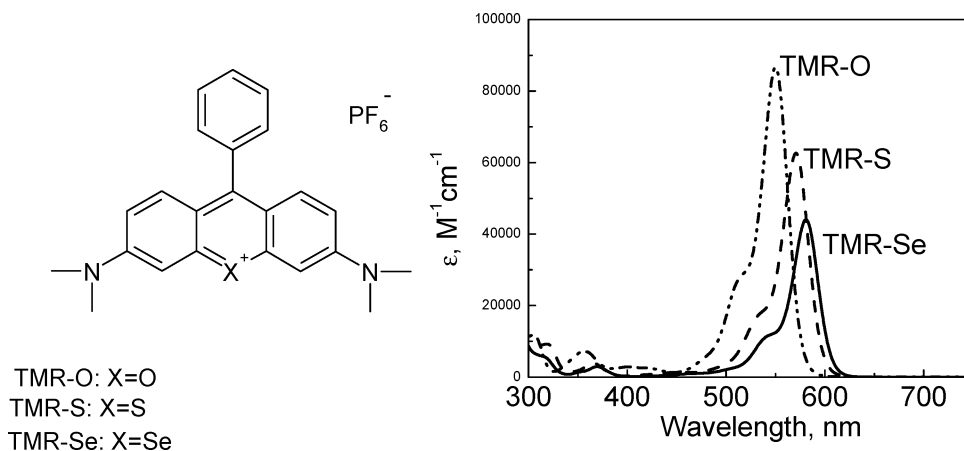


Figure 1. Chemical structures of the tetramethylrosamine dyes (TMR-X) and their absorption spectra in methanol solution at 293 K.

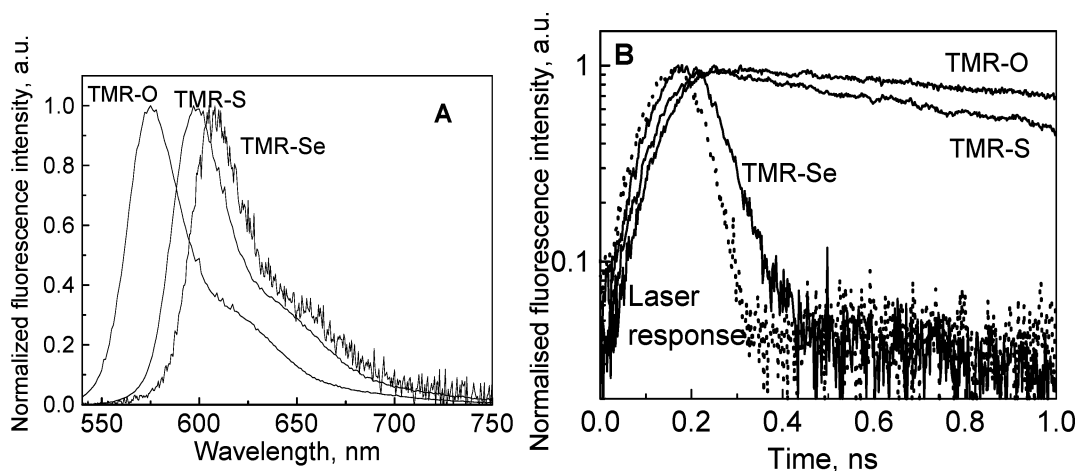


Figure 2. Fluorescence characteristics of the TMR-X dyes in methanol at a concentration of 5 μM and at 293 K. (A) Steady-state fluorescence spectra with excitation at 532 nm. (B) Fluorescence decay with pulse excitation at 405 nm.

rescence decay measurements at room temperature were done using a Hamamatsu C4334 streak camera. In this case, the excitation source used was an argon ion laser pumped, regeneratively amplified Ti:Sapphire laser (REGA 9000 from Coherent Inc.) operating at a 250-kHz repetition rate. To get the exciting wavelength, the 810-nm output of the amplifier was doubled using a type-I BBO crystal.

A SPEX 270M spectrometer (Jobin Yvon) equipped with an InGaAs photodetector (Electrooptical Systems Inc., USA) was used for acquisition of singlet oxygen emission spectra and dye emission in the near-IR spectral range. A diode-pumped solid-state laser (Verdi, Coherent) at 532 nm was the excitation source. The sample solution in a quartz cuvette was placed directly in front of the entrance slit of the spectrometer, and the exciting laser beam was directed at 90° relative to the collection of emission. Long-pass filters, 538AELP or 950 LP (Omega Optical, USA), were used to attenuate the excitation laser light and fluorescence from dyes in case of the study of singlet oxygen phosphorescence and TMR-Se phosphorescence decay.

To register phosphorescence decay, an Infinium oscilloscope (Hewlett-Packard) was used. Measurements at 77 K were performed with dye/methanol solutions in glass cells placed in a dewar flask filled with liquid nitrogen. For 77-K phosphorescence decay studies, an optical chopper, MC1000 (Thorlabs, USA), operating at 50 Hz was applied to produce excitation pulses from a CW excitation source (532 nm). The phosphorescence emission was detected by the InGaAs photodetector

of the SPEX 270M spectrometer and was directly recorded on the Infinium oscilloscope. To obtain phosphorescence decay at 293 K, a fast silicon photodiode was used. In this case, a detector was straight in front of the cuvette at 90° to the exciting laser beam. Long-pass filters, 538AELP and 700LP, were placed directly on the face of the detector.

Removal of the molecular oxygen from solutions at room temperature was realized by N₂ bubbling at the time of the experiment.

Results and Discussions

The absorption spectra of TMR-X dyes are presented in Figure 1. The shapes of the absorption bands for all molecules in the series are typical for rhodamine and rosamine molecules with peaks at 550, 571, and 581 nm for TMR-O, TMR-S, and TMR-Se, respectively. An increase in the size of the chalcogen atom in the rosamine chromophore gives two major changes in absorbance. (1) A bathochromic shift is observed (an energy difference of 670 cm⁻¹ between TMR-O and TMR-S and 300 cm⁻¹ between TMR-S and TMR-Se). This is in agreement with the changes in the oxidation and the reduction potentials and consequent narrowing in the HOMO-LUMO gap as the chalcogen atom, incorporated in the chromophore, becomes heavier.¹⁷ (2) The molar extinction coefficient becomes smaller with the increasing size of the chalcogen atom. The latter is apparently associated with a decrease in the oscillator strength of the S₀ → S₁ transition as a result of substitution of the heavier atom. According to Nijegorodov and

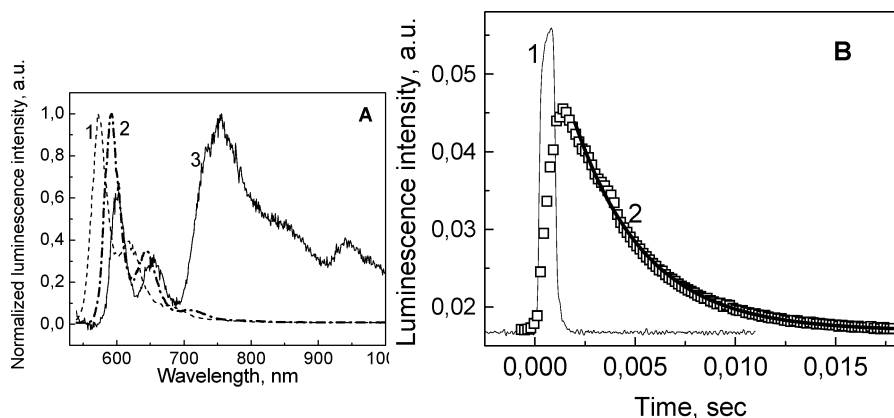


Figure 3. (A) Luminescence of TMR-O (1), TMR-S (2), and TMR-Se (3) at 77 K in a MeOH glass. (B) Excitation light pulse (1) and decay of TMR-Se phosphorescence ($\lambda = 750$ nm) at 77 K (2). A monoexponential fit with $\tau \approx 3$ ms is shown.

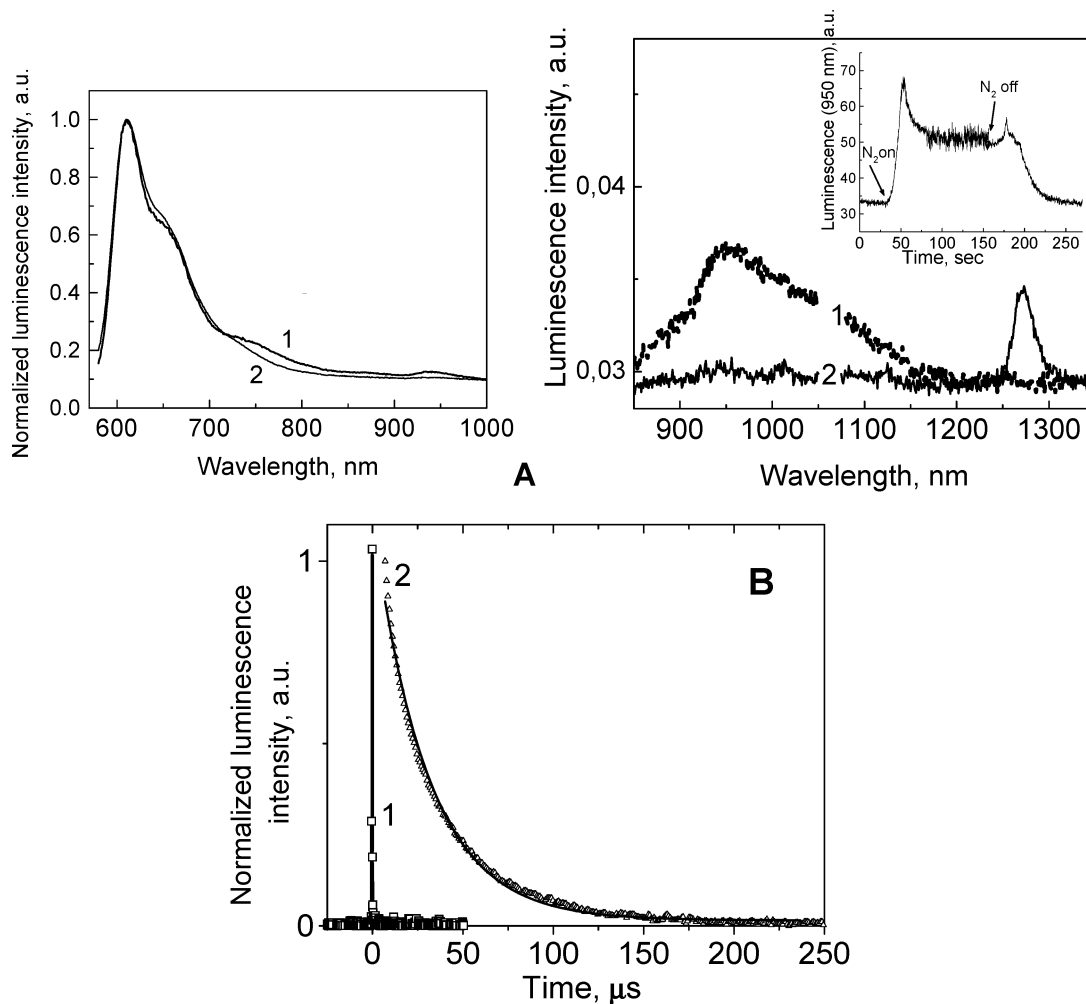


Figure 4. (A) Luminescence from TMR-Se methanol solution at 293 K with nitrogen bubbling (1) and without nitrogen bubbling (2). Spectra in the left panel were normalized. The right panel represents a different spectral range and scale. In the inset, the dependence of TMR-Se luminescence at 950 nm on bubbling of N_2 through the dye solution is shown. (B) Decay of TMR-O (1) and TMR-Se (2) luminescence from methanol solutions with nitrogen bubbling at 293 K. A monoexponential fitting ($\tau_{ph} \approx 34 \mu$ s) is shown (2).

Mabbs,²² along with an increase in the relative rate of intersystem crossing, the introduction of a heavy atom may also decrease the oscillator strength for the $S_0 \rightarrow S_1$ transition. Such an effect has been noted with chalcogenapyrylium dyes¹⁷ and with brominated rhodamines,¹⁴ but it is far more pronounced as a 50% reduction in ϵ , as the oxygen atom in TMR-O is replaced by the selenium atom of TMR-Se (Figure 1).

The fluorescence spectra of the TMR-X dyes (λ_{max} (TMR-O) = 575 nm, λ_{max} (TMR-S) = 599 nm, λ_{max} (TMR-Se) =

608 nm) follow the same trend observed in the absorption spectra, with similar red shifts (part A of Figure 2). The emission bands are a mirror image of the fluorescence bands if one compares Figure 1 and Figure 2A. However, the fluorescence intensities differ dramatically, with fluorescence quantum yields (γ_F) of 0.84 ± 0.02 , 0.44 ± 0.01 , and 0.009 ± 0.001 , for TMR-O, TMR-S, and TMR-Se, respectively. These large changes in γ_F can be attributed to a strong heavy-atom effect from S and, especially, from Se atoms incorporated in the xanthylum

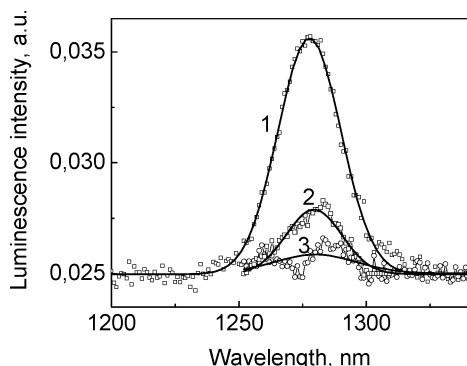


Figure 5. Luminescence of $^1\text{O}_2$ sensitized with TMR-Se (1), TMR-S (2), and TMR-O (3), with excitation at 532 nm in MeOH solutions. Gaussian fits are shown for every band. Concentrations of all solutions were adjusted to give OD = 0.4 at 532 nm.

ring. Distinct differences in the rosamine series also manifest themselves in the fluorescence decay of the TMR-X dyes (part B of Figure 2). The decay lifetime, τ , for TMR-O fluorescence is 2.1 ns, which is close, for example, to that of rhodamine B, also having dimethylamino groups.²³ For TMR-S, τ is 1.5 ns, and for TMR-Se, τ is approximately 50 ps. These data, as well as the values of fluorescence quantum yields for TMR-X dyes, denote a strong perturbation due to the Se atom in the xanthylium ring.

Population of the triplet level, T_1 , was directly observed in the luminescence spectra of TMR-X dyes in MeOH glasses at 77 K. As shown in Figure 3A, emission from TMR-Se displays fluorescence with a maximum close to that observed at room temperature as well as strong luminescence with the main peak in the range of 750 nm. This emission has a monoexponential decay with $\tau_{\text{ph}77} \approx 3$ ms (Figure 3B). This long-lived emission is consistent with the assumption that it is phosphorescence from TMR-Se. The S_1-T_1 energy gap, as calculated from the two maxima, is close to the 3100-cm^{-1} value reported for various rhodamine dyes.¹⁶ We observed no phosphorescence at 77 K from either TMR-O or TMR-S (Figure 3).

We also examined room-temperature phosphorescence²⁶ in the MeOH solution of TMR-Se. The solution was degassed by bubbling nitrogen through the TMR-Se solution in the cuvette at the time of spectral acquisition. Room-temperature phosphorescence was observed with nitrogen purging (Figure 4A) and the phosphorescence lifetime, $\tau_{\text{ph}293}$, was determined to be $\sim 34 \mu\text{s}$ (Figure 4B). The phosphorescence lifetime is nearly 2 orders of magnitude shorter at 293 K relative to 77 K and suggests that nonradiative decay of the triplet competes with phosphorescence at room temperature.

While acquiring the phosphorescence spectra of TMR-Se at 293 K, we could observe a clear and elegant illustration of the quenching of the triplet state by molecular oxygen. In the right panel of Figure 4A, the phosphorescence spectra are presented. When nitrogen purging is present, the TMR-Se phosphorescence is displayed (1). When the nitrogen purging is off, the TMR-Se phosphorescence disappears and, at the same time, an emission in the range of 1250–1300 nm (2)

appears and rises up, which is identical to singlet oxygen luminescence with a characteristic maximum at 1270 nm.²⁷

In Figure 5, the luminescence spectra of $^1\text{O}_2$ produced by sensitization with TMR-Se (1), TMR-S (2), and TMR-O (3) are shown. An observable signal is present for all three dyes including TMR-O. By use of Rose Bengal as a reference (singlet oxygen yield in methanol: $Q = 0.80$),²⁸ singlet oxygen yields (Q) were determined for the TMR-X dyes. They are 0.08 ± 0.01 , 0.21 ± 0.01 , and 0.87 ± 0.02 for TMR-O, TMR-S, and TMR-Se, respectively.

It is possible to estimate triplet quantum yields (γ_T), using the expressions¹²

$$\gamma_T = k_{\text{ISC}}/(k_r + k_{\text{nr}}) \quad (1)$$

$$k_{\text{nr}} = k_{\text{IC}} + k_{\text{ISC}} + k_{[\text{O}_2]} = (1 - \gamma_F)/\tau \quad (2)$$

where $k_r = \gamma_F/\tau$ is the radiative rate constant, k_{nr} is the nonradiative rate constant, k_{IC} is the rate constant of internal conversion, k_{ISC} is the rate constant for intersystem crossing, and $k_{[\text{O}_2]}$ is the rate constant for quenching by molecular oxygen presented in the solution. Because of the short fluorescence lifetime, τ , in our samples, one can neglect the quenching of fluorescence by molecular oxygen and assume that $k_{[\text{O}_2]} \approx 0$. Thus, to obtain γ_T , one should first determine k_{IC} or k_{ISC} . However, triplet quantum yields can also be estimated using the obtained data for singlet oxygen yields, assuming that the increased triplet yield correlates with an increase in the singlet oxygen yield. Of course, quenching of the triplet state by other pathways is still possible. Schmidt and coauthors have shown^{13,23–25} a correlation between the efficiency of singlet oxygen sensitization (S_Δ) and triplet energy (E_T) for the photosensitizers with negligible charge transfer (CT) interactions. The efficiency of singlet oxygen sensitization is defined as

$$S_\Delta = Q/(\gamma_T^{\text{O}_2} \gamma_T) \quad (3)$$

where $\gamma_T^{\text{O}_2}$ is the efficiency of quenching of the triplet excited state by molecular oxygen. For $\pi\pi^*$ triplet sensitizer with $E_T \approx 168 \text{ kJ mol}^{-1}$, corresponding values for S_Δ and $k_{\text{T}[\text{O}_2]}$ (rate constant for quenching of the first excited triplet state (T_1) by molecular oxygen) are ~ 0.9 and $\leq 3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, respectively.^{23,24} The values of T_1 energies of TMR-X dyes fall in the same range as the triplet energies for sensitizers reported in the literature.^{23,24} As deduced from Figure 3A, E_T for TMR-Se is $\approx 166 \text{ kJ mol}^{-1}$, and it should be slightly higher for TMR-O and TMR-S, taking into account spectral shifts. Furthermore, the structures are cationic, which excludes the possibility of strong CT interactions responsible for quenching by O_2 . On the basis of these arguments, we could apply the values of S_Δ and $k_{\text{T}[\text{O}_2]}$, taken from earlier references,^{23,24} to the TMR-X dyes. As seen in Figure 4, a highly efficient quenching of TMR-Se phosphorescence by air oxygen is experimentally observed, which would suggest that $\gamma_T^{\text{O}_2} = 1.0$. Moreover, the value of $\gamma_T^{\text{O}_2} = 1.0$ is commonly assumed for the air-saturated ($[\text{O}_2] \approx 2 \times 10^{-3} \text{ M}$)^{12,13} solutions of $\pi\pi^*$ triplet

TABLE 1: Molar Extinction Coefficient ϵ at the Absorption Peak, Fluorescence Quantum Yields γ_F , Fluorescence Decay τ , Radiative Rate k_r , Nonradiative Rate k_{nr} , Rate of Internal Conversion k_{IC} , Rate of Intersystem Crossing k_{ISC} , Triplet Yield γ_T and Singlet Oxygen Yield Q for Studied TMR-X Dyes

	ϵ (λ_{max} , nm), $10^3 \text{ M}^{-1} \text{ cm}^{-1}$	γ_F	τ , ns	k_r , 10^8 s^{-1}	k_{nr} , 10^7 s^{-1}	k_{ISC} , 10^7 s^{-1}	k_{IC} , 10^7 s^{-1}	γ_T	Q
TMR-O	$86.6 \pm 1(550)$	0.84 ± 0.02	2.1 ± 0.1	4	7.6	4.3	3.3	0.09	0.08 ± 0.02
TMR-S	$62.6 \pm 1(571)$	0.21 ± 0.01	1.5 ± 0.1	2.9	37	15	22	0.23	0.21 ± 0.01
TMR-Se	$44 \pm 1(581)$	0.009 ± 0.001	0.05 ± 0.01	1.8	1980	1938	42	0.97	0.87 ± 0.02

sensitizers in contrast to $n\pi^*$ triplet states, taking into account longer triplet-state lifetimes for $\pi\pi^*$ triplet states.^{13,23,24} By use of values $S_{\Delta} = 0.9$ and $\gamma_T^{02} = 1.0$ and equations 1–3, we could estimate triplet yields and other photophysical parameters for the TMR–X series as compiled in Table 1.

Conclusions

1. The photophysical properties of a series of chalcogenoxanthylum dyes (TMR–O, TMR–S, TMR–Se) were studied in which the substitution of S and Se for O in the xanthylum chromophore gave increased intersystem conversion $S_1 \rightarrow T_1$ and, consequently, higher quantum yields for the generation of singlet oxygen.

2. Accordingly, TMR–S and especially TMR–Se displayed decreased quantum yields for fluorescence and shortened radiative lifetimes, while at the same time proportionally larger increases in the rates of intersystem crossing lead to higher triplet yields (0.23 and 0.97, correspondingly). TMR–Se displays efficient phosphorescence at 77 K and at room temperature when the solution is purged of oxygen.

3. A high singlet oxygen yield for TMR–Se (0.87) suggests its use as an efficient photosensitizer for the generation of singlet oxygen. We have already achieved photodynamic action in our cellular studies. This work will be reported separately.

4. A series of TMR–X dyes can provide useful models for the calculation of the heavy-atom effect on spin–orbit coupling in multiatomic molecules.

Acknowledgment. This work was supported in part by the Directorate of Chemistry and Life Sciences of the Air Force Office of Scientific Research and in part by the National Institutes of Health (Grant CA69155 to M.R.D.). We thank Dr. M. D. Furis for help with TMR–X fluorescence lifetime measurements and Dr. A. Kuzmin for help with room-temperature phosphorescence decay measurements.

References and Notes

- (1) Peterson, O. G.; Tuccio, S. A.; Snively, B. B. *Appl. Phys. Lett.* **1970**, *17*, 245–247.
- (2) Ali, M.; Moghaddasi, J.; Ahmed, S. A. *J. Appl. Opt.* **1990**, *29*, 3945–3949.

- (3) Karstens, T.; Kobs, K. *J. Phys. Chem.* **1980**, *84*, 1871–1872.
- (4) Preininger, C.; Mohr, G. J.; Klimant, I.; Wolfbeis, O. S. *Anal. Chim. Acta* **1996**, *334*, 113–123.
- (5) Liu, J.; Diwu, Z.; Leung, W.-Y.; Lu, Y.; Patch, B.; Haugland, R. P. *Tetrahedron Lett.* **2003**, *44*, 4355–4359.
- (6) Menzel, R.; Thiel, E. *Chem. Phys. Lett.* **1998**, *291*, 237–243.
- (7) Shea, C. R.; Sherwood, M. E.; Flotte, T. J.; Chen, N.; Scholz, M.; Hasan, T. *Cancer Res.* **1990**, *50*, 4167–4172.
- (8) Haugland, R. P. *Molecular probes. Handbook of fluorescent probes and research products*, 9th ed.; Molecular Probes, Inc.: Eugene, OR, 2002.
- (9) Shea, C. R.; Chen, N.; Wimberly, J.; Hasan, T. *Cancer Res.* **1989**, *49*, 3961–3965.
- (10) Petrat, F.; Pindiur, S.; Kirsch, M.; de Groot, H. *J. Biol. Chem.* **2003**, *278*, 3298–3307.
- (11) Hasan, T. *Photodynamic therapy: basic principles and clinical applications*; Marcel Dekker: New York, 1992.
- (12) Birks, J. B. *Photophysics of Aromatic molecules*, Wiley-Interscience: London-New York-Sydney-Toronto, 1970.
- (13) Schweitzer, C.; Schmidt, R. *Chem. Rev.* **2003**, 1685–1757.
- (14) Pal, P.; Zeng, H.; Durocher, G.; Girard, D.; Li, T. C.; Gupta, A. K.; Giasson, R.; Blanchard, L.; Gaboury, L.; Balassy, A.; Turmel, C.; Laperriere, A.; Villeneuve, L. *Photochem. Photobiol.* **1996**, *63*, 161–168.
- (15) Villeneuve, L.; Pal, P.; Durocher, G.; Migneault, D.; Girard, D.; Giasson, R.; Balassy, A.; Blanchard, L. and Gaboury, L. *J. Fluoresc.* **1996**, *6*, 209–219.
- (16) Stracke, F.; Heupel, M.; Thiel, E. *J. Photochem. Photobiol., A* **1999**, *126*, 51–58.
- (17) Detty, M. R.; Merkel, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 3845–3855.
- (18) Reindl, S.; Penzkofer, A. *Chem. Phys.* **1996**, *211*, 431–439.
- (19) Gratz, H.; Penzkofer, A. *Chem. Phys.* **2001**, *263*, 471–490.
- (20) Samoc, A.; Samoc, M.; Luther-Davies, B. *Pol. J. Chem.* **2002**, *76*, 345–358.
- (21) Brennan, N. K.; Donnelly, D. J.; Detty, M. R. *J. Org. Chem.* **2003**, *68*, 3344–3347.
- (22) Nijegorodov, N.; Mabbs, R. *Spectrochim. Acta A* **2001**, *57*, 1449–1462.
- (23) Bodesheim, M.; Schütz, M.; Schmidt, R. *Chem. Phys. Lett.* **1994**, *221*, 7–14.
- (24) Schweitzer, C.; Mehrdad, Z.; Noll, A.; Grabner, E.-W.; Schmidt, R. *J. Phys. Chem. A* **2003**, *107*, 2192–2198.
- (25) Mehrdad, Z.; Schweitzer, C.; Schmidt, R. *J. Phys. Chem. A* **2002**, *106*, 228–235.
- (26) Donkerbroek, J. J.; Elzas, J. J.; Gooijer, C.; Frei, R. W.; Velthorst N. H. *Talanta* **1981**, *28*, 717–723.
- (27) Khan, A. U.; Kasha, M. *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 6047–6050.
- (28) Demas, J. N.; Harris, E. W.; McBride, R. P. *J. Am. Chem. Soc.* **1977**, *99*, 3547–3551.