Inorg. Chem. **2007**, *46*, 395–402

Inorganic Chemistry

Article

Single- and Two-Photon Properties of a Dye-Derivatized Roussin's Red Salt Ester ($Fe_2(\mu\text{-}RS)_2(NO)_4$) with a Large TPA Cross Section

Stephen R. Wecksler,[†] Alexander Mikhailovsky,[†] Dmitry Korystov,[†] Fabian Buller,[†,2] Ramamurthi Kannan,[‡] Loon-Seng Tan,[*,‡] and Peter C. Ford[*,†]

Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106-9510, and Polymer Branch, Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson Air Force Base, Ohio 45433-7750

Received April 28, 2006

The synthesis, characterization, photochemistry, and two-photon photophysical properties of a new dye-derivatized iron sulfur nitrosyl cluster $Fe_2(\mu\text{-}RS)_2(NO)_4$ (**AFX-RSE**, RS = 2-thioethyl ester of *N*-phenyl-*N*-(3-(2-ethoxy)phenyl)-7-(benzothiazol-2-yl)-9,9-diethyl-fluoren-2-yl-amine) were investigated. Under continuous photolysis, **AFX-RSE** decomposes with modest quantum yields ($\Phi_{diss} = (4.9 \pm 0.9) \times 10^{-3}$ at $\lambda_{irr} = 436$ nm) as measured from the loss of the nitrosyl bands in the IR absorbance spectrum. Nitric oxide (NO) was qualitatively demonstrated to be photochemically produced via single-photon excitation through the use of an NO-specific electrode. Steady-state luminescence measurements have shown that **AFX-RSE** fluorescence is about 88% quenched relative to the model compound **AF-tosyl**. This is attributed to a relatively efficient energy transfer from the excited states of the antenna chromophores to the dinuclear metal center, with the subsequent production of NO. In addition, the two-photon absorption (TPA) cross sections (δ) were measured for the AF-chromophores via the two-photon excitation (TPE) photoluminescence technique using a femtosecond excitation source. The TPA cross section of **AFX-RSE** was found with this technique to be $\delta = 246 \pm 8$ GM (1 GM = 10^{-50} cm^4 s photon^{-1} molecule^{-1}).

Introduction

Nitric oxide (NO, nitrogen monoxide) has important roles in mammalian biology, including functions in the cardiovascular system, neurotransmission, and immune response.[3] Furthermore, NO is known to be a γ-radiation sensitizer and, as such, may be useful in the treatment of certain hypoxic tumors.[4] These properties have generated considerable interest in developing drugs for the delivery of NO for the treatment of disease, with several in clinical use.[5] One limitation of such drugs is that thermally activated NO release is nonspecific, and large localized concentrations cannot be achieved without undesired cardiovascular implications. An alternative strategy to address this issue would be one in which a thermally stable precursor is triggered photochemically to release NO on demand.[6] In these contexts, we have been concerned with the development of thermally stable metal

---

[*] To whom correspondence should be addressed. E-mail: ford@chem.ucsb.edu (P.C.F.), Loon-Seng.Tan@wpafb.af.mil (L.-S.T.).

[†] University of California, Santa Barbara.

[‡] Wright-Patterson Air Force Base.

(1) Taken in part from the Ph.D. dissertation of S. R. Wecksler, University of California, Santa Barbara, 2006.

(2) Visiting undergraduate researcher from the University of Muenster, Germany.

(3) (a) Ignarro, L. J.; Buga, G. M.; Wood, K. S.; Byrns, R. E.; Chaudhuri, G. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9265. (b) *Vasodilation: Vascular Smooth Muscle, Peptides, Autonomic Nerves and Endothelium*; Furchgott, R. F., Ed.; Raven Press: New York, 1988. (c) Palmer, R. M. J.; Ferrige, A. G.; Moncada, S. *Nature* **1987**, *327*, 524. (d) Palmer, R. M. J.; Ashton, D. S.; Moncada, S. *Nature (London)* **1988**, *333*, 664−666. (e) Azuma, H.; Ishikawa, M.; Sekizaki, S. *Br. J. Pharmacol.* **1986**, *88*, 411−415. (f) Radomski, M. W.; Palmer, R. M. J.; Moncada, S. *Br. J. Pharmacol.* **1987**, *92*, 181−187. (g) Steuhr, D. J.; Marletta, M. A. *J. Immunol.* **1987**, *139*, 518−525. (h) Iyengar, R.; Stuehr, D. J.; Marletta, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 6369−6373. (i) *Nitric Oxide: Biology and Pathobiology*; Ignarro, L. J., Ed.; Academic Press: San Diego, 2000.

(4) (a) Howard-Flanders, P. *Nature (London)* **1957**, *180*, 1191. (b) Thomlinson, R. H.; Gray, L. H. *Br. J. Cancer* **1995**, *9*, 539−549. (c) Powers, W. E.; Tolmach, L. J. *Nature (London)* **1963**, *197*, 710−711. (d) Mitchell, J. B.; Wink, D. A.; DeGraff, W.; Gamson, J.; Keefer, L. K.; Krishna, M. C. *Cancer Res.* **1993**, *53*, 5845−5848.

(5) (a) Fung, H.-L. *Annu. Rev. Pharmacol. Toxicol.* **2004**, *44*, 67−85. (b) Katsuki, S.; Arnold, W. P.; Murad, F. *J. Cyclic Nucleotide Res.* **1977**, *3*, 239−247. (c) Marsh, N.; Marsh, A. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 313−319. (d) Cena, C.; Lolli, M. L.; Lazzarato, L.; Guaita, E.; Morini, G.; Coruzzi, G.; McElroy, S. P.; Megson, I. L.; Fruttero, R.; Gasco, A. *J. Med. Chem.* **2003**, *46*, 747−754.

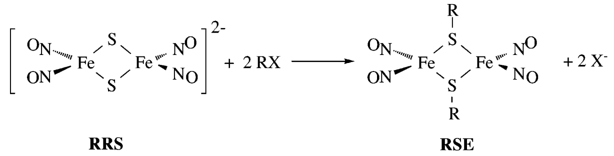
(6) (a) Ford, P. C.; Bourassa, J.; Miranda, K. M.; Lee, B.; Lorkovic, I.; Boggs, S.; Kudo, S.; Laverman, L. *Coord. Chem. Rev.* **1998,** *171*, 185−202. (b) Pavlos, C. M.; Xu, H.; Toscano, J. P. *Curr. Top. Med. Chem.* **2005**, *5*, 635−645.

10.1021/ic0607336 CCC: $37.00

nitrosyl and nitrito complexes that are capable of the photochemical generation of NO for therapeutic applications.[7−10]

A property of considerable importance for the use of photoactivated agents *in vivo* is the ability to absorb wavelengths of light where tissue penetration is maximized and that are sufficiently energetic to labilize the metal−nitrosyl bond selectively. For mammalian tissue, near-infrared (NIR; $\lambda = 800-1100$ nm) light has the greatest transmission.[11] Therefore, one focus of our current research is the development of compounds that can be subjected to single- or two-photon excitation (SPE or TPE) by NIR light, resulting in the photochemical production of NO. TPE is especially interesting given that this allows access to excited states that would require near-ultraviolet wavelengths if activation were to be achieved by SPE. Another key advantage of TPE for pro-drug activation is spatial selectively achieved from the intensity-squared dependence ($A \sim I_0^2$, where I_0 is the intensity of the incident light and A is the absorption rate) of the two-photon absorption (TPA) process, since the highest probability of TPA is at the focal point of a focused excitation beam.[12] For analogous reasons, TPE has drawn attention in strategies for photodynamic therapy involving singlet oxygen generation in tissue via organic dye photosensitization and for the photochemical “uncaging” of bioactive substances.[13]

Recent studies at the University of California at Santa Barbara (UCSB) have been concerned with the photochemical properties of Roussin’s red salt esters ($Fe_2(\mu\text{-}RS)_2(NO)_4$; RSEs), which can be prepared from Roussin’s red salt (RRS) (eq 1).[14−19] The simple esters (R = $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2OH$, $-CH_2CH_2SO_3$) undergo photochemical decomposition with moderate quantum yields ($\Phi_d = 0.02-0.13$) when irradiated with near-UV light and release ∼4 mol of NO per mole of cluster decomposed.[14] However, the absorptivity of the iron sulfur nitrosyl chromophore is quite low at longer visible wavelengths, thus limiting potential *in vivo* applications. In this context, dye-derivatized compounds, such as **Fluor-RSE** (RS = 2-thioethyl ester of fluorescein)[17,18] and **PPIX-RSE** (RS = bis(2-thioethyl)diester protoporphyrin IX),[15,16] were prepared with the goal of imparting specific optical properties to improve the light harvesting ability and the photochemical response to longer wavelength light. Both **PPIX-RSE** and **Fluor-RSE** proved to be much more photochemically effective than the simple RSE complexes at longer wavelengths, since the dye chromophores dramatically improve the light collecting efficiency for longer visible wavelengths under continuous photolysis conditions where single-photon excitation (only) would be expected.[15,17]



(1)

Although attaching antennae to the iron sulfur nitrosyl cluster improved the light harvesting efficiency, the quantum yield for photodecomposition for these clusters decreases at longer irradiation wavelengths.[15,17] An alternative strategy to address this issue is to use TPA in the NIR to achieve up-conversion to higher energy state(s) from which the desired photochemistry results. This was first achieved qualitatively with **PPIX-RSE**, for which TPE with 810 nm femtosecond pulses leads both to weak emission from the PPIX chromophore and to photochemical NO labilization.[16] While this provided “proof of concept”, the poor solubility of **PPIX-RSE** and the recognition that porphyrins are very modest TPA chromophores ($\delta = 2$ GM for PPIX, 1 GM = 10^{-50} cm^4 s photon^{-1} molecule^{-1})[20] led us to develop other compounds such as **Fluor-RSE** with fluorescein antennae.[17] Fluorescein has well-characterized photophysical behavior,

---

(7) (a) Hoshino, M.; Ozawa, K.; Seki, H.; Ford, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 9568−9575. (b) Lorkovic, I. M.; Miranda, K. M.; Lee, B.; Bernhard, S.; Schoonover, J. R.; Ford, P. C. *J. Am. Chem. Soc.* **1998**, *120*, 11674−11683. (c) Laverman, L.; Ford, P. C. *J. Am. Chem. Soc.* **2001**, *123*, 11614−11622. (d) Joseph, C.; Ford, P. C. *J. Am. Chem. Soc.* **2005**, *127*, 6737−6743.

(8) (a) Works, C. F.; Ford, P. C. *J. Am. Chem. Soc.* **2000**, *122*, 7592−7593. (b) Works, C. F.; Jocher, C. J.; Bart, G. D.; Bu, X.; Ford, P. C. *Inorg. Chem.* **2002**, *41*, 3728−3739.

(9) (a) De Leo, M.; Ford, P. C. *J. Am. Chem. Soc.* **1999**, *121*, 1980−1981. (b) De Leo, M.; Ford, P. C. *Coord. Chem. Rev.* **2000**, *208*, 47−59. (c) DeRosa, F.; Bu, X.; Ford, P. C. *Inorg. Chem.* **2005**, *44*, 4157−4165. (d) DeRosa, F.; Bu, X.; Pohaku, K.; Ford, P. C. *Inorg. Chem.* **2005**, *44*, 4166−4174.

(10) (a) Bourassa, J.; DeGraff, W.; Kudo, S.; Wink, D. A.; Mitchell, J. B.; Ford, P. C. *J. Am. Chem. Soc.* **1997,** *119,* 2853−2860. (b) Bourassa, J.; Lee, B.; Bernard, S.; Schoonover, J.; Ford, P. C. *Inorg. Chem.* **1999**, *38*, 2947−2952. (c) Bourassa, J.; Ford, P. C. *Coord. Chem. Rev.* **2000**, *200−202*, 887−900.

(11) (a) Dougherty, T. J.; Marcus, S. L. *Eur. J. Cancer* **1992**, *28A*, 1734−1742. (b) Master, B. R.; So, P. T.; Gratton, E. *Biophys. J.* **1997**, *72*, 2405−2412.

(12) (a) Denk, W.; Strickler, J. H.; Webb, W. W. *Science* **1990**, *248*, 73−76. (b) König, K. *J. Microsc. (Oxford)* **2000**, *200*, 83−104. (b) So, P. T. C.; Dong, C. Y.; Masters, B. R.; Berland, K. M. *Annu. Rev. Biomed. Eng.* **2000**, *2*, 399−429. (c) Zhou, W.; Kuebler, S. M.; Braun, K. L.; Yu, T.; Cammack, J. K.; Ober, C. K.; Perry, J. W.; Marder, S. R. *Science* **2002**, *296*, 1106−9.

(13) (a) Dougherty, T. J.; Kaufman, J. E.; Goldbarb, A.; Weishaupt, K. R.; Boyle, D.; Mittleman, A. *Cancer Res.* **1978**, *38*, 2628. (b) Bodaness, R. S.; Heller, D. F.; Krasinski, J.; King, D. S. *J. Biol. Chem.* **1986**, *261*, 12098−12101. (c) Fischer, W. G.; Partridge, W. P., Jr.; Dees, C.; Wachter, E. A. *Photochem. Photobiol.* **1997**, *66*, 141−155. (d) Bhawalkar, J. D.; Kumar, N. D.; Zhao, C.-F.; Prasad, P. N. *J. Clin. Lasers Med. Surg.* **1997**, *15*, 201−204. (e) Jaconi, M.; Pyle, J.; Bortolon, R.; Ou, J.; Clapham, D. *Curr. Biol.* **1997**, *7*, 599−602. (f) Lipp, P.; Niggli, E. *J. Physiol.* **1998**, *508*, 801−809. (g) Brown, E. B.; Webb, W. W. *Methods Enzymol.* **1998**, *291*, 356−380. (h) Furuta, T.; Wang, S. S.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193−1200. (i) Frederiksen, P. K.; Jørgensen, M.; Ogilby, P. R. *J. Am. Chem. Soc.* **2001**, *123*, 1215−1221. (j) Dichtel, W. R.; Serin, J. M.; Edder, C.; Fréchet, J. M. J.; Matuszewski, M.; Tan, L.-S.; Ohulchanskyy, T. Y.; Prasad, P. N. *J. Am. Chem. Soc.* **2004**, *126*, 5380−5381. (k) Nikolenko, V.; Yuste, R.; Zayat, L.; Baraldo, L. M.; Etchenique, R. *Chem. Commun.* **2005**, 1752−1754.

(14) Conrado, C. L.; Bourassa, J.; Egler, C.; Wecklser, S.; Ford, P. C. *Inorg. Chem.* **2003**, *42*, 2288−2293.

(15) Conrado, C. L.; Wecksler, S.; Egler, C.; Magde, D.; Ford, P. C. *Inorg. Chem.* **2004**, *43*, 5543−5549.

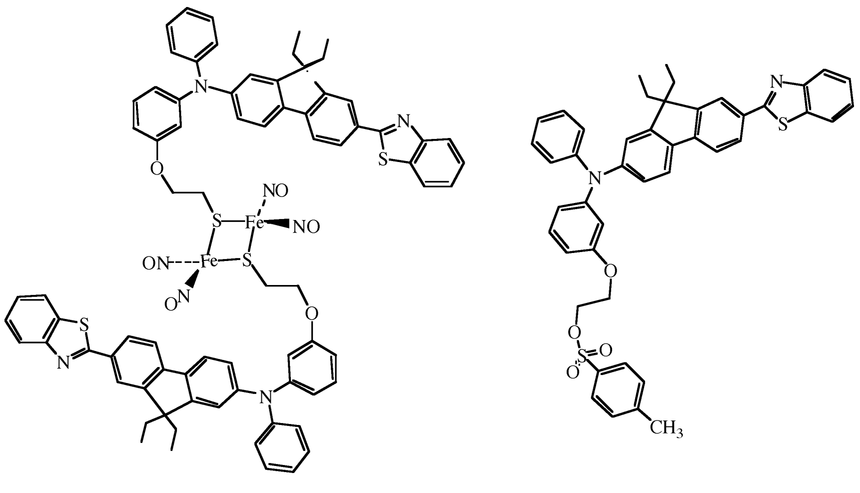
(16) Wecksler, S. R.; Mikhailovsky, A.; Ford, P. C. *J. Am. Chem. Soc.* **2004**, *126*, 13566−13567.

(17) Wecksler, S. R.; Hutchinson, J.; Ford, P. C. *Inorg. Chem.* **2006**, *45*, 1192−1200.

(18) Wecksler, S. R.; Mikhailovsky, A.; Korystov, D.; Ford, P. C. *J. Am. Chem. Soc.* **2006**, *128*, 3831−3837.

(19) (a) Roussin, F. Z. *Ann. Chim. Phys.* **1858**, *52*, 285. (b) Butler, A. R.; Glidewell, C.; Li, M.-H. *Adv. Inorg. Chem.* **1988**, *32*, 335−393.

(20) Goyan, R. L.; Cramb, D. T. *Photochem. Photobiol.* **2000**, *72*, 821−827.



AFX-RSE

AF-tosyl (AF343b)

Figure 1. Formulas for **AFX-RSE** and **AF-tosyl**.

is water soluble, and has a moderate TPA cross section ($\delta = 38$ GM at 782 nm for fluorescein in pH 11 aqueous solution).[21]

Like the case of **PPIX-RSE**, the excitation of **Fluor-RSE** with pulsed femtosecond light at 800 nm results in frequency up-conversion and weak emission from the antennae along with the photochemical production of NO.[18] The TPA cross section measured for **Fluor-RSE** is $\delta = 63$ ($\pm$ 7) GM at 800 nm, about twice that of the free chromophore. Furthermore, intensity dependence studies showed the quantity of NO generated using 100 femtosecond pulses of 800 nm light to follow a quadratic relationship. This result confirmed the operation of a two-photon mechanism under these conditions, leading to the generation of higher energy excited states from which nonradiative processes populate the reactive states responsible for NO labilization. These findings represent significant progress toward the goal of using NIR two-photon excitation for photochemical NO production but remain limited by the need to develop systems with larger TPE cross sections δ.

There has been significant progress in recent years in developing compounds with large TPA cross sections,[22,23] and it has been shown that δ is largely a function of the transition dipole moment resulting from an intramolecular charge-transfer excitation.[23] This has driven the development of structural motifs, such as paracyclophane derivatives,[22d] fused diporphyrins,[22e] bis(styryl)benzene/oligo-stilbene derivatives,[22g−i] and the diphenylaminofluorene-based compounds known as the AF chromophores,[22b,24,25] having donor−π-acceptor−π-donor or acceptor−π-donor−π-acceptor properties that may enhance dipole-moment changes, thus giving larger δ values.[23] The AF chromophores (e.g., Figure 1) demonstrate synthetic versatility owing to the nature of the attached linker functionality and show significant promise for photonic applications. In the present manuscript, we describe the synthesis, photochemistry, and two-photon photophysical properties of a new dye-derivatized RSE for which the light harvesting antennae are such AF chromophores.

---

(21) Webb, W. W.; Xu, C. *J. Opt. Soc. Am. B* **1997**, *13*, 481−491.

(22) (a) Meyers, F.; Marder, S. R.; Pierce, B. M.; Brédas, J.-L. *J. Am. Chem. Soc.* **1994**, *116*, 10703−10714. (b) Baur, J. W.; Alexander, M. D., Jr.; Banach, M.; Denny, L. R.; Reinhardt, B. A.; Vaia, R. A. *Chem. Mater.* **1999**, *11*, 2899−2906. (c) Marder, S. R.; Gorman, C. B.; Meyers, F.; Perry, J. W.; Bourhill, G.; Brédas, J.-L.; Pierce, B. M. *Science* **1994**, *265*, 632−635. (d) Woo, H. Y.; Hong, J. W.; Mikhailovsky, A.; Korystov, D.; Bazan, G. C. *J. Am. Chem. Soc.* **2005**, *127*, 820−821. (e) Kim, D. Y.; Ahn, T. K.; Kwon, J. H.; Kim, D.; Ikeue, T.; Aratani, N.; Osuka, A.; Shigeiwa, M.; Maeda, S. *J. Phys. Chem. A* **2005**, *109*, 2996−2999. (f) Rumi, M.; Ehrlich, J. E.; Heikal, A. A.; Perry, J. W.; Barlow, S.; Hu, Z.; McCord-Maughon, D.; Parker, C. T.; Rockel, H.; Thayumanavan, S.; Marder, S. R.; Beljonne, D.; Brédas, J.-L. *J. Am. Chem. Soc.* **2000**, *122*, 9500−9510. (g) Belfield, K. D.; Morales, A. R.; Kang, B.-S.; Hales, J. M.; Hagan, D. J.; Van Stryland, E. W.; Chapela, V. M.; Percino, J. *Chem. Mater.* **2004**, *16*, 4634−4641. (h) Katan, C.; Terenziani, F.; Mongin, O.; Werts, M. H. V.; Porres, L.; Pons, T.; Mertz, J.; Tretiak, S.; Blanchard-Desce, M. *J. Phys. Chem. A* **2005**, *109*, 3024−3037. (i) Drobizhev, M.; Karotki, A.; Dzenis, Y.; Rebane, A.; Suo, Z.; Spangler, C. W. *J. Phys. Chem. B* **2003**, *107*, 7540−7543.

(23) Albota, M.; Beljonne, D.; Bredas, J. L.; Ehrlich, J. E.; Fu, J. Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M. D.; Marder, S. R.; McCord-Maughon, D.; Perry, J. W.; Rockel, H.; Rumi, M.; Subramaniam, G.; Webb, W. W.; Wu, X. L.; Xu, C. *Science* **1988**, *281*, 1653−1656.

(24) (a) Tan, L.-S.; Kannan, R.; Matuszewski, M. J.; Khur, I. J.; Feld, W. A.; Dang, T. D.; Dombroskie, A. G.; Vaia, R. A.; Clarson, S. J.; He, G. S.; Lin, T.-C.; Prasad, P. N. *Proc. SPIE—Int. Soc. Opt. Eng.* **2003**, *4797*, 171−178. (b) He, G. S.; Lin, T.-C.; Dai, J.; Prasad, P. N.; Kannan, R.; Dombroskie, A. G.; Vaia, R. A.; Tan, L.-S. *J. Chem. Phys.* **2004**, *120*, 5275−5284. It should be pointed out that inherent in the nonlinear absorption (transmission) measurement using either nanosecond or picosecond laser pulses is the assumption that the two-photon process is the predominant mechanism responsible for the observed intensity-dependent nonlinear absorption. However, it is now well recognized that the strong TPA process increases molecular populations considerably in excited states, and thus a secondary process, i.e., the cascaded one-photon absorption from the excited states, may greatly enhance the observed nonlinear absorption of the input laser beam. Since it is difficult to separate these two contributions through the nonlinear absorption measurement in these time regimes, the term “effective TPA cross section” is used to describe the apparent δ values under these excitation conditions for the two-photon chromophore. The effective cross section of a TPA chromophore can be more than 2 orders of magnitude larger than the intrinsic cross section obtained via a femtosecond masurement, depending on the type of technique (transmission or fluorescence method) used.

(25) (a) Rogers, J. E.; Slagle, J. E.; McLean, D. G.; Sutherland, R. L.; Sankaran, B.; Kannan, R.; Tan, L.-S.; Fleitz, P. A. *J. Phys. Chem. A* **2004**, *108*, 5514−5520. (b) Sutherland, R. L.; Brant, M. C.; Heinrichs, J.; Rogers, J. E.; Slagle, J. E.; McLean, D. G.; Fleitz, P. A. *J. Opt. Soc. Am. B* **2005**, *22*, 1939−1948.

Experimental Section

Materials. Tetrahydrofuran (THF) used for synthesis and photochemical measurements was distilled under dinitrogen from sodium and benzophenone. Dichloromethane (DCM) was distilled under N_2 from calcium hydride (CaH_2). Silica gel 60 (Geduran 60 Å) was purchased from EMC sciences. Gases were purchased from Praxair Inc. and used as received unless specified. High grade argon used for inert atmosphere preparations was passed through a Drierite/molecular sieve (4 Å) column to remove water. Deoxygenated solutions were prepared by entraining with dinitrogen or argon for ~1 min/mL. Inert atmosphere work was performed on a Schlenk line or in an argon-filled glove box (VAC Nexus model). Deaerated solutions were prepared via three freeze−pump−thaw cycles.

The synthesis and characterization of AF-341,[24] AF-343 (**AF-Br**),[24] and Roussin's red salt ($Na_2[Fe_2(\mu\text{-}S)_2(NO)_4]$, RRS)[10] are described elsewhere. AF-343-b (**AF-tosyl**) was made in two steps from AF-341 (*N*-phenyl-*N*-(3-hydroxyphenyl)-7-(benzothiazol-2-yl)-9,9-diethylfluoren-2-ylamine). AF-341 was first alkylated with ethylene carbonate to produce the phenoxy−ethanol intermediate, which was then reacted with *p*-toluenesulfonyl chloride (tosyl chloride) in pyridine to get the tosylate, AF-343-b (**AF-tosyl**). Fluorescein dye was purchased from Acros Organics and used as received.

***N*-Phenyl-*N*-(3-(2-hydroxyethoxy)phenyl)-7-(benzothiazol-2-yl)-9,9-diethylfluoren-2-ylamine.** A mixture of 10.76 g of AF-341 (20 mmol), 2.76 g of potassium carbonate (20 mmol), and dimethylformamide (DMF, 100 mL) was heated to 60 °C, and then 3.54 g of ethylene carbonate (40 mmol) was added. After 24 and 30 h, two additional portions each of potassium carbonate (1.38 g, 10 mmol) and ethylene carbonate (1.8 g, 20 mmol) were added. After a total of 48 h at 60 °C, the mixture was diluted with water, and the separated greenish yellow solids (11.48 g) were collected. These solids were dissolved in a 5% ethyl acetate−toluene mixture and passed through a column of silica gel. The residue that was left behind on evaporation of the solvents was recrystallized from a mixture of isopropanol and methanol to afford 10.91 g (94% yield) of the product, mp 143−145 °C. Another recrystallization raised the mp to 145.1−146.5 °C. MS: *m*/*z* 582 (M^+). ^1H NMR ($CDCl_3$) δ ppm: 0.38 (t, 6H), 1.19 (1H), 1.90−2.15 (m, 4H), 3.88−3.99 (m, 4H), 6.57−8.10 (m, 19H). ^{13}C NMR ($CDCl_3$) δ ppm: 8.61, 30.93, 32.63, 56.41, 61.34, 69.03 (sp^3C), 108.68, 110.01, 116.63, 119.05, 119.39, 121.01, 121.41, 121.52, 122.88, 123.08, 123.57, 124.49, 124.98, 126.28, 127.28, 129.27, 129.90, 131.46, 134.86, 135.52, 144.45, 147.61, 147.87, 149.14, 150.67, 152.05, 154.15, 159.48, 168.84. Anal. Calcd for $C_{38}H_{34}N_2O_2S$: C, 78.32; H, 5.88; N, 4.81; S, 5.50. Found: C, 77.96; H, 6.03; N, 4.55; S, 5.56.

***N*-Phenyl-*N*-(3-(2-tosylethoxy)phenyl)-7-(benzothiazol-2-yl)-9,9-diethylfluoren-2-ylamine AF-343-b (AF-tosyl).** To a solution of (3.5 g, 6 mmol) *N*-phenyl-*N*-(3-(2-hydroxyethoxy)phenyl)-7-(benzothiazol-2-yl)-9,9-diethylfluoren-2-ylamine in pyridine (21 mL), cooled in an ice-bath, 4-toluenesulfonyl chloride (2.2 g, 12.1 mmol) was added. The mixture was then allowed to warm to room temperature. After 6 h, the mixture was diluted with water, and the separated orange solids (4.74 g) were collected. The product mixture was chromatographed over silica gel and eluted with a 3:1 toluene−heptane mixture. The desired product was obtained in 73% yield (3.24 g) after recrystallization from a 1:3 toluene−heptane mixture, mp 153.4−155.7 °C. MS: *m*/*z* 736 (M^+). ^1H NMR ($CDCl_3$) δ ppm: 0.37 (t, 6H), 1.90−2.35 (m, 4H), 2.40 (s, 3H), 4.03−4.06 (m, 2H), 4.28−4.31 (m, 2H), 6.45−8.10 (m, 23H). ^{13}C NMR δ ppm: 8.82, 21.85, 32.81, 56.63, 65.80, 68.24 (sp^3C), 108.72, 110.21, 117.10, 119.29, 119.64, 121.24, 121.66, 121.75, 123.09, 123.37, 123.76, 124.68, 125.21, 125.49, 126.51, 127.50, 128.23, 128.42, 129.23, 129.51, 130.01, 130.11, 131.67, 132.93, 135.04, 135.79, 144.64, 145.13, 147.77, 148.03, 149.33, 150.91, 152.31, 154.29, 159.09, 169.04 (sp^2 C). Anal. Calcd for $C_{45}H_{40}N_2O_4S_2$: C, 73.34; H, 5.47; N, 3.80; S, 8.70. Found: C, 73.46; H, 5.54; N, 3.64; S, 8.58. Also recovered from this chromatographic separation were (i) unreacted starting alcohol, 0.2 g (6%), mp 138.6−142.1 °C, and (ii) chloroethoxy AF-341, 0.15 g (4% yield), mp 140.8−142.2 °C, MS *m*/*z* 600, 602 (M^+). The latter appears to have been generated from the reaction between **AF-tosyl** and the byproduct chloride ion.

Bis[(*μ*-*S*,*μ*-*S′*)-*N*-phenyl-*N*-(3-(2-ethoxy)phenyl)-7-(benzothiazol-2-yl)-9,9-diethylfluoren-2-ylamine-thioethylester]tetranitrosyl diiron (AFX-RSE). A three-neck flask (100 mL) covered in aluminum foil and equipped with a stir bar and rubber septa was charged with a solution containing 0.5 g (0.00068 mol) of *N*-phenyl-*N*-(3-(2-tosylethoxy)phenyl)-7-(benzothiazol-2-yl)-9,9-diethylfluoren-2-ylamine (**AF-tosyl**) in approximately 50 mL of dry, distilled deoxygenated THF. This solution was blanketed with argon and stirred until the compound was fully dissolved, and then 0.165 g (0.00034 mol) of solid recrystallized $Na_2[Fe_2S_2(NO)_4]\cdot 8H_2O$ (RRS) was added under inert gas. This mixture was refluxed under argon for approximately 4 h, after which the solution was checked by IR and TLC to ensure that the reaction had gone to completion. At this point, the solution was allowed to cool to room temperature, and then the solvent was removed via rotary evaporation. The remainder was washed with deoxygenated water and was then evaporated to complete dryness on the rotary evaporator.

The product was further purified via chromatography on silica gel with a DCM/hexanes mixture as the eluent. The first band collected from the column was evaporated to dryness and found to contain not only the desired material but also a small amount of unreacted **AF-tosyl** precursor. This material was further purified by flash chromatography on Geduran 60 Å silica gel (63 μm) with a DCM/hexanes mixture as the eluent. The first band collected from the column no longer contained the unreacted precursor, only the desired **AFX-RSE**. The fraction contained 420 mg (0.000294 mol) of material, giving a yield of 43.3%. The compound was further purified by recrystallization via diethyl ether diffusion into DCM under an inert atmosphere. The compound was characterized by UV−vis, IR, low-resolution ESI-MS, ^1H NMR, and C, H, and N elemental analysis. UV−vis (THF) (λ_{max} in nm (ϵ in M^{-1} cm^{-1})): 242 (5.52×10^4), 308 (4.93×10^4), 394 (7.81×10^4). IR (THF) (ν_{NO} in cm^{-1} (ϵ in M^{-1} cm^{-1})): 1752 (5.16×10^3), 1777 (4.97×10^3). ESI-MS (20% CH_2Cl_2 in CH_3OH): *m*/*z* 1427 ($[(C_{38}H_{33}N_2OS)_2Fe_2S_2(NO)_4] + H^+)^+$, 714 ($[(C_{38}H_{33}N_2OS)_2Fe_2S_2(NO)_4] + 2H^+)^{2+}$. ^1H NMR (in *d*-THF, δ = ppm, J = Hz): 0.40 (m, 12H), 1.90−2.25 (m, 8H), 3.42 (t, 5.6 Hz, 4H), 4.27 (m, 4H), 6.65−8.15 (m, 38H). Anal. Calcd for $(C_{38}H_{33}N_2OS)_2Fe_2S_2(NO)_4$: C, 63.96; H, 4.63; N, 7.85. Calcd for $(C_{38}H_{33}N_2OS)_2Fe_2S_2(NO)_4\cdot CH_2Cl_2$: C, 61.45; H, 4.46; N, 7.35. Found (Marine Science Institute analytical lab at UCSB): C, 61.38; H, 4.65; N, 6.77. Our experience with other RSEs is that they typically give low N values despite considerable other experimental support for their overall purity.

Instruments. UV−vis absorption spectra were recorded using a HP8572 diode array spectrophotometer or a Shimadzu dual beam UV-2401 PC spectrophotometer and using high quality quartz UV−vis cells (NSG Precision Cells Inc., Starna Cells) with 1 cm path lengths. IR spectra were obtained on a BioRad FTS 60 SPC 3200 FTIR spectrometer. Solution IR spectra were recorded in sealed cells (Reflex Analytical Inc, International Crystal Labs) with a fixed path length of 0.05 or 0.02093 cm and CaF_2 windows with Teflon spacers and stoppers. Detection of NO in solution was performed using an amiNO-700 electrode from Innovative Instruments, Inc. (Tampa, FL) capable of quantitatively detecting nanomolar amounts in aqueous solution. The electrode was calibrated from 0 to 400 nM in NO concentration via injections of known quantities of nitrite standards into acidified solutions in the presence of a reducing agent.

Steady-state fluorescence spectra were recorded using a Spex Fluorolog2 spectrofluorimeter with a 150 W xenon lamp excitation source. The resulting emission was collected at 90°. The excitation and emission beams were passed through SPEX 1681 Singlemate monochromators to isolate the appropriate wavelengths, and the emitted light was recorded with a Hamamatsu R928-A water-cooled photomultiplier tube (PMT) configured for single-photon counting and interfaced with a computer running Spex DM3000f software. The emission spectra were corrected for PMT response and deviations in lamp intensity by the ratio method using rhodamine-6G as a standard. Samples were prepared in four-sided 1-cm path length quartz cuvettes (Starna cells) such that the absorbance at the excitation wavelength was less than 0.1. The photoluminescence quantum yield for **AF-tosyl** was determined by comparing the integrated fluorescent emission intensity referenced to fluorescein (in buffered solution at pH 11: $\epsilon = 7.69 \times 10^4$ M^{-1} cm^{-1} at $\lambda = 490$ nm, $\Phi = 0.93$).[26] The optical absorbance spectra (<0.1) were matched at the excitation wavelength ($\lambda_{ex} = 390$ nm), and the corresponding integrated fluorescence intensity was measured (from $\lambda_{mon} = 400-750$ nm) using a Photon Technology International fluorimeter.

Photochemical Experiments. The optical train for continuous photolysis experiments used a 200 W Hg arc lamp in an Oriel model 66033 lamp housing equipped with an Oriel model 68910 light intensity controller as the excitation source. The light was passed through a water-filled cell for IR filtration, through neutral density filters, and through the appropriate interference filter (366 or 436 nm) to isolate the desired irradiation wavelength. The intensities at $\lambda_{irr} = 366$ or 436 nm were determined by using the ferrioxalate actinometer.[27] Quantum yields (Φ) were calculated by monitoring changes in the FTIR spectra of the sample solutions in an IR cell (CaF_2 windows, 2.0 mm path length Teflon spacer, and Teflon stopper) custom designed to allow actinometry and photochemical measurements to be made under identical conditions. The samples were stirred continuously with a mini stir flea and kept from exposure to extraneous light. Known concentrations were prepared in aerated solutions and irradiated for various periods of time.

TPE Fluorescent Measurements. These were accomplished in the UCSB Optical Characterization Facility. Samples were excited with a tightly collimated (~120 μm diameter), high-intensity laser beam, and the corresponding up-converted fluorescence was detected at 90°. TPE was achieved using a mode-locked Ti–sapphire laser (Spectra Physics Tsunami) operating with a repetition rate of 80 MHz and providing ~6 nJ, 100 femtosecond excitation pulses. The emitted light from the sample was collected by a high numerical aperture lens and routed into the spectrometer (Acton SpectraPro-300) equipped with a thermoelectrically cooled CCD camera (Roper Scientific PIXIS:400). Solutions were irradiated for ~5 s with a central excitation wavelength (λ_{ex}) of 800 nm, and the TPE-induced fluorescence was monitored over the range 400–750 nm.

Two-Photon Absorption Cross Section (δ) Measurements. These utilized the TPA photoluminescence excitation technique described by Xu and Webb[21] and employed the experimental setup described in the previous paragraph. The measurements of the integrated fluorescence intensity (480–700 nm) enabled us to determine δ according to the equation[18]

$$\delta = \frac{\Phi_{ref}\delta_{ref}c_{ref}P_{ref}{}^2 I}{\Phi c P^2 I_{ref}} K \tag{2}$$

where the subscript ref denotes values obtained in the reference sample under identical experimental conditions. The correction factor K is derived from the refractive indices for the solvents used to prepare the samples ($K = n^2/n_{ref}^2$). I and I_{ref} are the integrated fluorescence intensities, P and P_{ref} are the excitation powers employed, c and c_{ref} are the concentrations, and Φ and Φ_{ref} are the fluorescence quantum yields under SPE for the sample and the reference, respectively. The reference standard utilized was fluorescein dye (10 μM in aqueous solution at pH 11 with $\Phi = 0.93$),[26] which has a δ value of 38 ± 9.7 GM at 782 nm under these conditions.[21]

Results and Discussion

Synthesis of AFX-RSE. The synthesis of **AFX-RSE** was achieved by refluxing a mixture of **AF-tosyl** or **AF-Br** with RRS in a 2:1 ratio under an inert atmosphere (eq 3). Within several hours, the reaction was complete, and once recovered as a solid, the resulting **AFX-RSE** was purified by flash chromatography on silica gel using DCM/hexanes as the eluent. The compound was isolated in a 43% yield. **AFX-RSE** is not soluble in aqueous solutions but is readily soluble in THF and DCM.

$$\text{AF-Y} + Na_2[Fe_2(\mu\text{-S})_2(NO)_4] \rightarrow \underset{(\textbf{AFX-RSE})}{Fe_2(\mu\text{-S-AF})_2(NO)_4} + \underset{(Y = Br^- \text{ or tosylate})}{2NaY} \tag{3}$$

The UV–vis and IR spectra of **AFX-RSE** are shown in Figure 2. The IR spectrum in THF displays three bands in the nitrosyl stretching frequency (ν_{NO}) at 1752 cm^{-1} ($\epsilon = 5.16 \times 10^3$ M^{-1} cm^{-1}), 1777 cm^{-1} (4.97×10^3 M^{-1} cm^{-1}), and 1815 cm^{-1} (w), quantitatively and qualitatively similar to the IR spectra of other Roussin's red salt esters.[14–17] The spectrum also displays a band at 1723 cm^{-1} that is also seen in the spectra of **AF-Br** and **AF-tosyl** and, thus, is characteristic of the AF chromophore. The UV–vis spectrum of the compound in THF closely resembles that for the AF precursors, with bands at 242 nm (5.52×10^4 M^{-1} cm^{-1}), 308 nm (4.93×10^4 M^{-1} cm^{-1}), and 394 nm (7.81×10^4 M^{-1} cm^{-1}). The strengths of the UV–vis bands for **AFX-RSE** are almost exactly twice those of the AF precursors, indicating that the attachment of the two chromophores to the red salt has resulted in a linear addition of the extinction coefficients. Thus, both the IR and the electronic spectra appear to be simply the sum of the spectra of the individual components of **AFX-RSE** (two AF chromophores plus one $Fe_2(\mu\text{-SR})_2(NO)_4$ cluster), so it is apparent that any electronic interaction between these centers must be weak.

Photochemical Studies. AFX-RSE is stable as a solid under an inert atmosphere and in the dark. It is moderately

---

(26) Sjöback, R.; Nygren, J.; Kubista, M. *Spectrochim. Acta, Part A* **1995**, *51*, L7–L21.

(27) (a) Calvert, J.; Pitts, J. *Photochemistry*; Wiley & Sons: New York, 1967. (b) Hatchard, C.; Parker, C. *Proc. R. Soc. London, Ser. A* **1956**, *235*, 518.

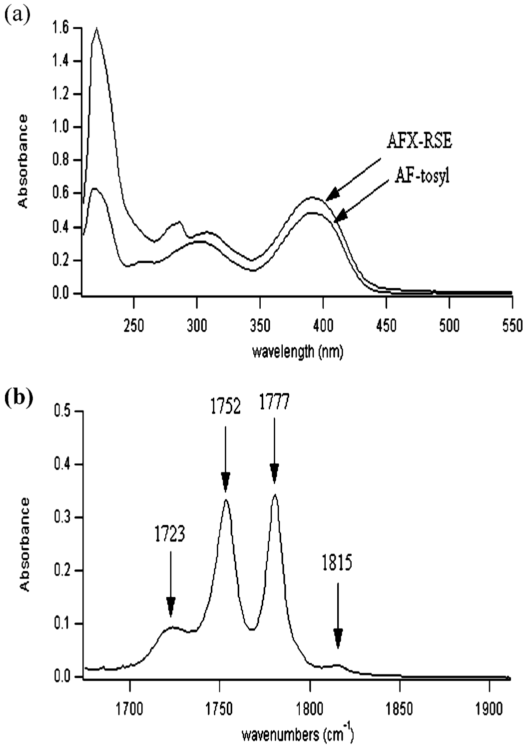


Figure 2. (a) Optical absorbance spectra of **AFX-RSE** (7.36 μM) and **AF-tosyl** (12.5 μM) in deoxygenated THF. (b) IR spectrum of **AFX-RSE** in THF.

stable in solution in the dark and under an inert atmosphere, but it undergoes photochemistry when irradiated in aerated solutions. However, such reactions could not be monitored by UV−vis spectroscopy because when a solution of **AFX-RSE** in aerated THF was subjected to continuous photolysis at $\lambda_{irr} = 366$ nm, there were no changes in the electronic spectrum. This behavior is quite different from that of **Fluor-RSE**, which did display significant changes,[17] but parallels that of **PPIX-RSE**, which did not.[15] This behavior was expected given that the optical spectra of **AFX-RSE** and **PPIX-RSE** both appear to be the algebraic sums of the spectra of the components of these supramolecular complexes, while that of **Fluor-RSE** demonstrated a surprising reduction in the $\pi \rightarrow \pi^*$ absorptions characteristic of the fluorescein antennae upon assembling the complex.

Therefore, like the case for **PPIX-RSE**, it was necessary to monitor the photodecomposition of **AFX-RSE** via changes in the absorptions due to the nitrosyl ν_{NO} bands in the IR spectrum (Figure 3). To make quantitative measurements without loss of volatile solvents that would occur during solution transfers, an IR cell was designed so that continuous photolysis and actinometry could be performed in the same cell. In such experiments, **AFX-RSE** (4.94×10^{-4} M) was dissolved in aerated solutions of distilled THF and irradiated for 30 s intervals with 366 or 436 nm light. Within the first 30 s of irradiation, decreases in the nitrosyl ν_{NO} bands at 1777 and 1751 cm^{-1} are apparent, and these bands diminish with each successive interval, resulting in total cluster decomposition within several minutes of irradiation. The quantum yields Φ_d for the photochemical decomposition at the irradiation wavelengths 366 and 436 nm were determined

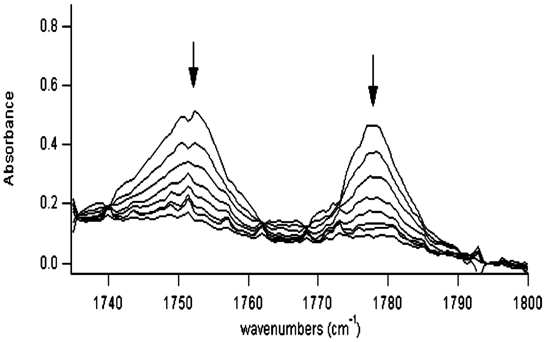
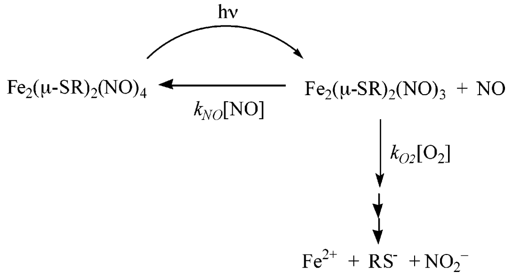


Figure 3. IR spectral changes upon continuous photolysis ($\lambda_{irr} = 436$ nm) of **AFX-RSE** (4.94×10^{-4} M) in an aerated THF solution. Successive spectra were recorded after 30 s irradiation intervals.

Scheme 1. Proposed Photochemical Mechanism for the RSE Complexes



to be 0.011 ± 0.001 ($I_a = 3.6 \times 10^{-5}$ einstein L^{-1} s^{-1}) and $(4.9 \pm 0.9) \times 10^{-3}$ ($I_a = 1.9 \times 10^{-5}$ einstein L^{-1} s^{-1}), respectively. Since the absorption spectrum of **AFX-RSE** is dominated by the dye chromophore at the excitation wavelengths, it appears that energy transfer from the initially excited AF antennae must account for most of the photoreactivity of this system.

Previously, we have shown that the simple RSE and dye-derivatized RSE complexes demonstrate analogous photochemistry under continuous photolysis with UV−vis light.[14,15,17] This indicates that excitation of the dye chromophores is followed by energy transfer to the iron sulfur nitrosyl cluster and population of the reactive excited states formed by direct excitation. Laser flash photolysis experiments (ns) of the simple RSE complexes have demonstrated that the primary photochemical step is the reversible labilization of NO (Scheme 1).[14] In deaerated solvent, this is followed by fast recombination ($k_{NO} = 1.1 \times 10^9$ M^{-1} s^{-1} for R = CH_2CH_2OH) to reform the parent complex. Under aerobic conditions, these intermediates are trapped by O_2, resulting in permanent photochemistry with the subsequent formation of various thiols, nitrite, and ferrous ion.

Like the case of **Fluor-RSE**,[17] the Φ_d for **AFX-RSE** photodecomposition at 436 nm is considerably larger than that recorded for **PPIX-RSE** at the same wavelength (λ_{irr}).[15] It should be noted, however, that the direct comparison of quantum yields for the net photochemistry of different Roussin's salts and esters has qualitative significance only, since these tend to be markedly dependent on the solvent medium and irradiation intensity I_a owing to the competition between the trapping of reaction intermediates by O_2 and the back-reaction of these with NO generated by photodissociation from the iron sulfur nitrosyl clusters.[14] For example,

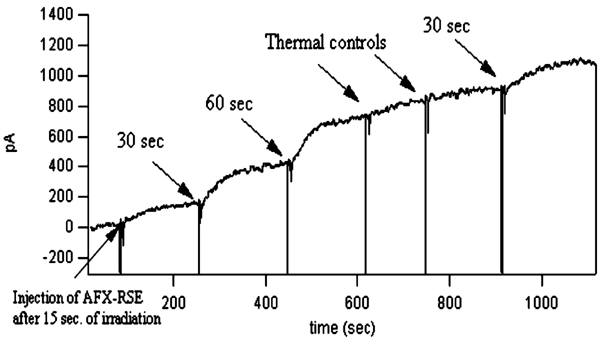


Figure 4. NO electrode response to injections of photolyzed **AFX-RSE** solutions.

higher I_a values would give higher steady-state NO concentrations and, hence, lower Φ_d values under analogous conditions. In this context, the Φ_d values reported above for **AFX-RSE** were obtained at a relatively high I_a and, hence, tend to underestimate the relative photoreactivity of that compound.

The loss of the ν_{NO} IR bands upon irradiation of **AFX-RSE** implies that the compound is undergoing photochemical decomposition with a concomitant release of NO. This was confirmed by continuous photolysis experiments with a NO-specific electrode for the direct detection of free NO. Solutions of **AFX-RSE** (6.5 μM) were prepared in distilled, aerated THF and placed in a quartz cuvette equipped with a stir flea. The **AFX-RSE** solutions were irradiated with 366 nm light, and 25 μL aliquots were withdrawn and injected into a conical vial containing the NO electrode immersed in 20 mL of deionized water. Upon injection of the photolyzed **AFX-RSE** aliquots into the solution in which the NO electrode is immersed, an immediate increase in signal was observed, indicating photochemical production of NO from the cluster. Figure 4 illustrates the NO electrode response for aliquots taken after 15, 30, and 60 s, followed by aliquots from a nonphotolyzed **AFX-RSE** solution as a thermal control and then an aliquot taken from the **AFX-RSE** solution photolyzed for another 30 s. The increased NO electrode currents clearly indicate the photolysis-induced NO release from the **AFX-RSE** solution and the absence of release from the thermal controls. Electrode calibration gives estimates of NO release in the photolysis aliquots of 1.5, 2.9, 3.3, and 2.2 nM, respectively. From a quantitative perspective, this experiment suffers from uncertainties regarding NO loss during solution transfers and, in general, associated with the electrochemical detection of NO.[29] Nonetheless, these control experiments clearly indicate that the photodecomposition of **AFX-RSE** is coupled to the release of NO. By analogy to studies with other RSE complexes,[14,15,17] the ratio of NO released to complex decomposed should be approximately four.

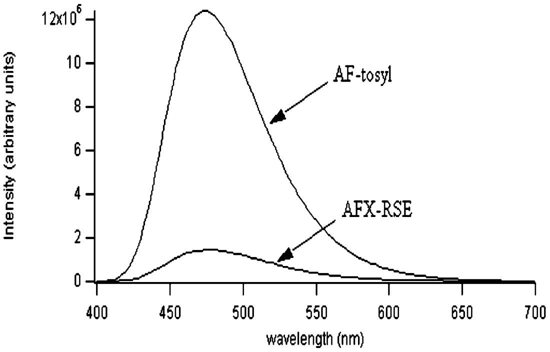


Figure 5. Steady-state luminescence spectra of **AFX-RSE** (0.69 μM) and **AF-tosyl** (1.29 μM) in deaerated solutions of THF with a matched absorbance at $\lambda_{exc} = 390$ nm.

Emission Studies. The continuous photolysis studies indicate that excitation of absorptions characteristic of the AF chromophore leads to NO release from **AFX-RSE**. A key question is, how effective is the quenching of the AF-specific excited state(s) by the iron sulfur nitrosyl core? Steady-state luminescence experiments were undertaken to investigate this question further. Solutions of **AFX-RSE** (0.69 μM) and **AF-tosyl** (1.29 μM) were prepared in dry, distilled THF under argon such that the absorbances (0.05) were equal at the excitation wavelength ($\lambda_{exc} = 390$ nm). The samples were irradiated at 390 nm, while the corresponding emission was monitored in the range $\lambda_{mon} = 400-750$ nm at a 90° angle. The photoluminescence (PL) quantum yield for the **AF-tosyl** derivative was measured by comparing the integrated emission intensity of the complex to that of fluorescein, and in this manner, Φ_{PL}(**AF-tosyl**) was calculated to be 1.00 ± 0.03. In comparison, the **AFX-RSE** emission intensity proved to be only about 12% of that for **AF-tosyl** (Figure 5); thus, some mechanism, presumably energy transfer to the iron sulfur nitrosyl cluster, is serving to quench ~88% of the fluorescence. If one assumes that the decreased emission of **AFX-RSE** is due solely to intramolecular energy transfer, then the rate constant for quenching (k_q) can be calculated from the relative emission according to $I_f/I_{f'} = 1 + k_q\tau$, where I_f and I_f' are the integrated emission intensities of **AFX-tosyl** and **AFX-RSE**, respectively, and τ is the fluorescence lifetime for the former ($\tau = 2.3$ ns).[25a] This gives $k_q = 3.2 \times 10^9$ s^{-1}.

Two-Photon Photophysics. The steady-state luminescence, CW photolysis, and NO electrode experiments demonstrate that the AF chromphores serve as light gathering antennae for **AFX-RSE** and that absorption of light by these chromophores results in energy transfer to the iron sulfur nitrosyl cluster and the photochemical production of NO. These experiments provided insight into the reactivity of excited states prepared by single-photon excitation, but the real interest in this compound lies in its two-photon photophysical properties. In this regard, similar deaerated solutions of **AFX-RSE** and **AF-tosyl** were subjected to excitation with 800 nm light using the output from the mode-locked Ti–sapphire laser (6 nJ pulses with half-widths of ~100 femtoseconds at 80 MHz pulse frequency). The respective solution concentrations (2.88×10^{-6} M and 9.21×10^{-6} M) were chosen to give approximately the same absorbance

---

(28) (a) Shen, Y. R. *The Principles of Non-linear Optics*; Wiley: New York, 1984. (b) Lin, S. H.; Fujimura, Y. F.; Neusser, H. J.; Schlag, E. W. *Multiphoton Spectroscopy of Molecules*; Academic Press: Orlando, FL, 1984. (c) Prasad, P. N.; Williams, D. J. *Introduction to Nonlinear Optical Effects in Molecules and Polymers*; Wiley: New York, 1991.

(29) (a) Wink, D. A.; Darbyshire, J. F.; Nims, R. W.; Saavedra, J. E.; Ford, P. C. *Chem. Res. Toxicol.* **1993**, *6*, 23. (b) Kudo, S.; Bourassa, J. L.; Boggs, S. E.; Sato, Y.; Ford, P. C. *Anal. Biochem.* **1997**, *247*, 193–202.

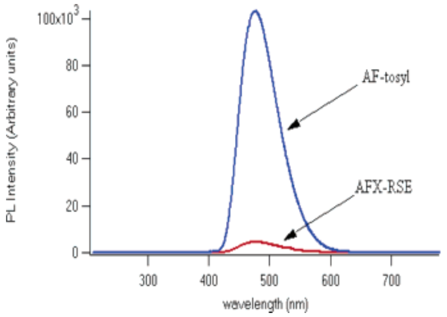


Figure 6. TPE PL spectra of **AFX-RSE** (2.88×10^{-6} M) and **AF-tosyl** (9.21×10^{-6} M) in deaerated solutions of distilled THF with 800 nm excitation.

(~0.25) at the wavelength (400 nm) corresponding to twice the frequency of the excitation source. The recorded spectrum of neither solution displays any absorption at the 800 nm excitation wavelength; however, both displayed measurable fluorescence at energy greater than the excitation energy, indicating the presence of a multiphoton absorption (Figure 6). While both samples displayed emission as a result of TPE under these conditions, the integrated photoluminescence intensity for **AFX-RSE** was ~5% of that of **AF-tosyl**. Since the two measurements were made under identical conditions, the ratio of the integrated TPE photoluminescence intensities is equal to the relationship described in eq 4. On the basis of the measured values of the respective Φ_{PL} and concentrations and the $I_{AFX-RSE}/I_{AF-tosyl}$ ratio, one can calculate the relative sensitivities of **AFX-RSE** and **AF-tosyl** toward TPE at 800 nm as $\delta_{AFX-RSE}/\delta_{AF-tosyl} = \sim 1.3$.

$$I_{AFX-RSE}/I_{AF-tosyl} = \frac{\Phi_{AFX-RSE}\delta_{AFX-RSE}c_{AFX-RSE}}{\Phi_{AF-tosyl}\delta_{AF-tosyl}c_{AF-tosyl}} \qquad (4)$$

In this context and for comparison, the TPA cross sections δ for **AFX-RSE** and **AF-tosyl** were determined directly by the TPA PL method using femtosecond excitation and fluorescein (at pH = 11, δ = 32 GM at 800 nm) as the reference standard.[21] Samples of **AFX-RSE** (~2.8 μM) and **AF-tosyl** (9.2 μM) were prepared in dry, distilled THF in the glove box under an inert atmosphere, and their TPA properties were measured immediately after sample preparation. Since the dye-derivatized RSE clusters photochemically decompose in aerated solution to products that are brighter luminophores, the samples were kept from exposure to extraneous light or oxygen, and each sample was used for only one data point in the δ spectrum to minimize any such photodegradation. A graph plotting the TPA cross sections measured in this manner (in GM) versus wavelength is shown in Figure 7. The ratio of the values $\delta_{AFX-RSE}/\delta_{AF-tosyl} = 1.76$ at 800 nm determined in this manner is somewhat larger than estimated by the experiment described above.

The "effective" TPA cross sections measured previously using the nanosecond nonlinear transmission method were found to be approximately 8000 GM,[24a] more than an order of magnitude higher than the values determined using the TPA PL method with femtosecond laser pulses described here. This discrepancy has been previously noted by others and was attributed to excited-state absorption when using the nanosecond excitation method.[24b] In our measurements using the TPA PL method, **AF-tosyl** demonstrated TPA cross sections of ~140 GM at 800 nm (Figure 7) and the δ for **AF-Br** was quite similar (150 GM). The ratio of the respective TPA cross sections is consistent with that found using the nanosecond nonlinear transmission method, albeit the magnitudes of the respective values are much lower than those found using the latter technique.[24b] The TPA cross section of **AFX-RSE** was found to be about twice that of the AF precursors, with a $\delta = 246 \pm 8$ GM at 800 nm. The origin of the enhanced molecular TPA cross section could be largely the result of an increase in the number density as each supramolecular **AFX-RSE** complex incorporates two TPA chromophore units. Nonetheless, **AFX-RSE** has a TPA cross section with NIR light four times larger than that of **Fluor-RSE** and this can be attributed to the significantly superior TPA properties of the AF chromophore.

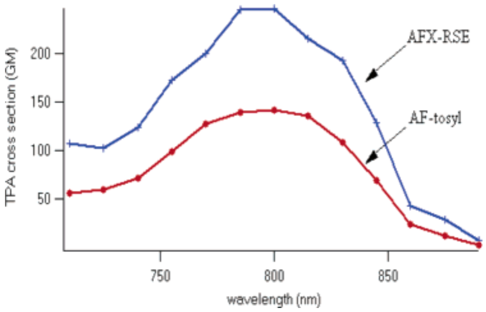


Figure 7. TPA cross section measurements of the AF compounds in THF.

Summary

A new dye-derivatized iron sulfur nitrosyl cluster **AFX-RSE** with a pendant chromophore designed to have a high two-photon absorption cross section has been prepared and characterized. Under continuous photolysis at 436 nm, this decomposes with a modest quantum yield and concomitant release of NO. Steady-state luminescence measurements show that the fluorescence from **AFX-RSE** is about 88% quenched relative to that from the model compound **AF-tosyl**, presumably as a result of energy transfer from the antenna excited states to the dinuclear metal center. Two-photon excitation of **AFX-RSE** with NIR light also leads to visible range fluorescence, and this was used to measure the TPA cross section of **AFX-RSE** ($\delta = 246 \pm 8$ GM) via the TPE photoluminescence technique using a femtosecond excitation source. These studies represent an incremental advance toward the very long-term goal of developing new materials for the photochemical delivery of the important bioregulator nitric oxide to specific targets using two-photon excitation.

Acknowledgment. These studies were supported by the National Science Foundation (CHE-0352650).

IC0607336