

## 7-Azabicyclo[2.2.1]heptane as a Unique and Effective Dialkylamino Auxochrome Moiety: Demonstration in a Fluorescent Rhodamine Dye

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Despite the spectacular successes that have been achieved using organic fluorophores in contemporary pure and applied science, the full potential of most luminescence-based technologies has yet to be realized largely because the goal of developing monomolecular chromophores that simultaneously have long-term photostabilities and high fluorescent efficiencies remains an unmet challenge. The limitations imposed by currently available fluorophores on cutting-edge experiments and technologies has led to calls for the development of improved, more versatile materials.<sup>1</sup>

Theoretical and experimental advances made in recent years in two disparate fields of chemistry have allowed us to use their combined databases and predictions to rationally design improved fluorescent reporter dyes. One of these sources of information arose from the continuing controversy associated with the TICT mechanism that has been proposed to explain dual fluorescence and enhanced rates of nonradiative decay in a wide variety of donor–acceptor (DA) dyes. This controversy has spawned a large amount of research into elucidating pertinent structure–function relationships that govern the photophysical behavior of these materials.<sup>2,3</sup> In addition, our effort was aided by the pioneering studies of the Nelsen and Mann groups aimed at understanding the physical chemistry of apex-N-substituted bicycloalkanes which have shown some members of this class of amines to be extraordinarily resistant to oxidative degradation.<sup>4</sup> In this communication we present the preliminary results of a study designed to test the idea that some of the deficiencies that have been linked to the use of ordinary dialkylamines as auxochrome moieties in DA fluorophores could be ameliorated, or, at the least, minimized, by the use of suitably configured, geometrically constrained amino groups. Specifically, using sulforhodamine 221SR as a model fluorophore and its tetramethyl analogue, TMSR, as a benchmark, we demonstrate the benefits of using 7-azabicyclo[2.2.1]heptane (7-azanorbornane) as an electron donating moiety in place of a more conventional dimethylamino group. These include (a) higher fluorescence quantum yields, (b) fluorescence lifetimes and quantum yields that are nearly independent of temperature, and (c) improved resistance to photo-oxidative dealkylation.

It has long been known that DA dyes bearing untethered dialkylamino donor moieties, such as the pendant dimethylamino groups in rhodamine TMSR, have lower fluorescent quantum yields than do analogues having lower degrees of substitution (NHR or NH<sub>2</sub>) or where amino group rotation is prevented by cyclization.<sup>5</sup> This increased propensity to undergo nonradiative decay has been found to correlate with both (i) steric requirements at the  $\alpha$ -position of the alkylamino substituents that promote donor–acceptor non-planarity and (ii) the ability of the donor group to stabilize a cationic charge as measured by the ionization potential of the pertinent amine.<sup>3</sup> It follows that the key to designing a more efficient

**Table 1.** Photophysical Data for 221SR and TMSR in Methanol

dye	$\lambda_{\text{abs}}$ (nm)	$\lambda_{\text{fl}}$ (nm)	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> )	$\Phi_{20\text{ }^\circ\text{C}}$	$\Phi_{60\text{ }^\circ\text{C}}^a$
221SR	548	567	112 000	0.95	0.95
TMSR	550	568	108 000	0.65	0.38

<sup>a</sup> Calculated by  $\Phi_{60\text{ }^\circ\text{C}} = \Phi_{20\text{ }^\circ\text{C}}(\tau_{60\text{ }^\circ\text{C}}/\tau_{20\text{ }^\circ\text{C}})(n_{60\text{ }^\circ\text{C}}^2/n_{20\text{ }^\circ\text{C}}^2)$ .

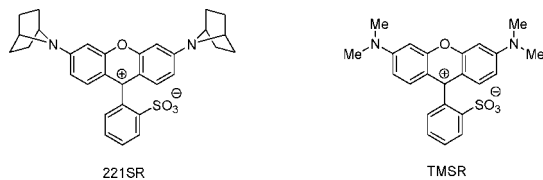
rhodamine than TMSR would be to find a replacement for the dimethylamino donor having diminished steric requirements and/or a higher ionization potential. These considerations led us to consider using 7-azanorbornane for this purpose for the following reasons: (1) With the use of 7-azanorbornyl benzene as a model to qualitatively gauge pertinent steric interactions, geometry optimization calculations [AM1] for both the neutral aniline and for its corresponding cation radical predicted norbornyl C<sub>1</sub>–N–C<sub>4</sub> bond angles of 95.0° and 97.3°, respectively, and a “closest approach” distance between the bridgehead hydrogen and ortho-benzene hydrogen atoms of 2.100 and 2.094 Å, respectively. These values correspond to considerably less steric interference than those calculated for *N,N*-dimethylaniline and its cation radical, which were predicted to have CH<sub>3</sub>–N–CH<sub>3</sub> bond angles of 113.4° and 118.2°, respectively, and methyl-benzene hydrogen atom separation distances of 1.609 and 1.594 Å, respectively. (2) Experimental adiabatic ionization potentials for 7-azanorbornane and dimethylamine are reported to be 8.40 and 8.24 eV, respectively.<sup>6</sup>

The targeted new dye 221SR was readily synthesized by heating 3,6-dichlorosulfofluoran with excess 7-azanorbornane. The dye was purified by flash chromatography and characterized by HPLC, TLC, and spectroscopic methods.

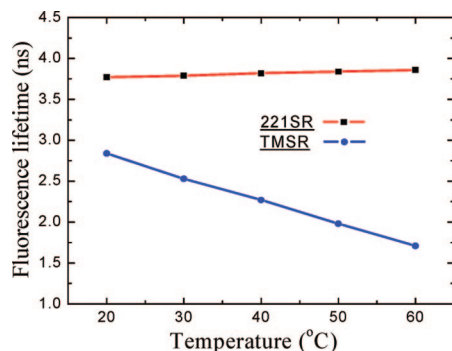
Spectroscopic measurements revealed that 221SR and TMSR have nearly identical absorption maxima, fluorescence maxima, extinction coefficients, and mirror image band shapes (Table 1). Thus, in these regards 7-azanorbornane behaves like a typical dialkylamino donor albeit with transition energies higher than would have been expected for a similarly sized bis-*n*-dialkyl analogue.<sup>7</sup> On the other hand, 221SR has an impressive fluorescence quantum yield of 0.95 which is significantly higher than the 0.65 value measured for its tetramethyl analogue. Moreover, despite being untethered, 221SR has an emission efficiency that rivals those of the very best rhodamines, regardless of the nature or flexibility of the donor groups.<sup>5</sup>

Unlike the typical dialkylamino DA dye, TMSR, whose fluorescence lifetime ( $\tau$ ) and quantum yield ( $\Phi$ ) decrease significantly with increasing temperature, the  $\tau$  for 221SR was found to increase and the  $\Phi$  to remain constant when the temperature was ramped from 20 to 60 °C (Table 1, Figure 2). These findings validate the high  $\Phi$  values measured for 221SR in light of the relationships:  $\Phi = k_r/(k_r + k_{nr})$  and  $\tau = 1/(k_r + k_{nr})$ , where  $k_r$  and  $k_{nr}$  are radiative and nonradiative rate constants, respectively.<sup>8</sup> In cases where  $k_r \gg k_{nr}$ , that is, where  $E_{\text{act}}$  for nonradiative decay is abnormally large,  $\tau$  should increase with temperature ( $k_r \propto n^2$  of the solvent)<sup>8</sup> whereas

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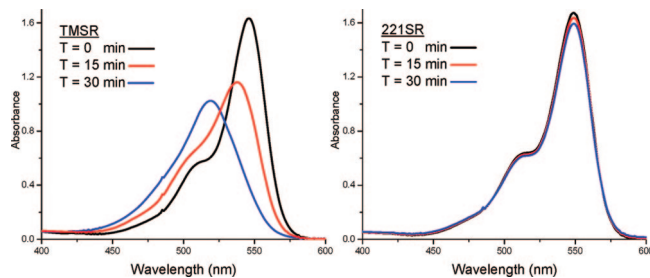
**Figure 1.** Structures of pertinent dyes depicted to emphasize connectivity.



**Figure 2.** Fluorescent lifetimes of 221SR and TMSR vs temperature in methanol (values reported in Supporting Information).

$\Phi$  should approach unity and be little affected by temperature, as is the case for 221SR.

For a fluorophore to be of practical value, it must be reasonably resistant to photodegradation. Although the light-induced decomposition of DA dyes can be complex, in this report we focus on the products that arise from the degradation of donor groups. In general, electron rich N-alkylated amino groups tend to undergo stepwise photo-oxidative dealkylation reactions which cause a blue shift in the absorption maximum of the dye.<sup>9</sup> A key step in this process is thought to be an  $\alpha$ -elimination reaction in an initially formed aminium radical ( $\text{CH}_3\text{NR}_2^{\cdot+}$ ) to give a reactive immonium ( $\text{CH}_2=\text{NR}_2^+$ ) salt.<sup>4</sup> Because a parallel sequence of reactions for a 7-azanorbornyl donor would require the formation of a highly energetic anti-Bredt immonium salt,<sup>10</sup> in accord with the findings of oxidation studies of several related azabicyclics,<sup>4</sup> 221SR should be resistant to this type of oxidative photodegradation. To test this hypothesis, we evaluated the photostabilities of 221SR and TMSR using relatively innocuous trifluoroethanol as solvent. Because preliminary studies in our laboratory and by others<sup>11,12</sup> found several tetramethylrhodamine derived dyes to be extremely resistant to photodegradation under aerobic conditions, our stability studies were conducted in a mildly alkaline, anaerobic environment, conditions designed to accelerate the photo-oxidative decomposition of the dyes;<sup>13,14</sup> illumination was provided by a 1.7 W, 514 nm CW laser. As is evident from inspecting Figure 3, 221SR is considerably more stable than TMSR; the former dye loses only a small amount of optical density at its absorption maximum without changing color during 30 min of intense illumination while, under identical conditions, the latter undergoes a dramatic decrease in its absorption maximum and a concomitant significant blue shift which is attributable to oxidation-induced demethylation. Thus, at least where photo-oxidative degradation is concerned, the use of 7-azanorbornane as an electron donor represents a new paradigm for



**Figure 3.** Photodegradation of dyes as a function of irradiation time.

achieving photostability in a rhodamine fluorophore and potentially in other members of the DA family of dyes as well.

In summary, to the best of our knowledge this investigation constitutes the first use of an apex-N-substituted azabicycloalkane as an amino auxochrome group in a donor–acceptor chromophore. We have demonstrated that the use of the 7-azanorbornyl moiety as the electron donating component in a rhodamine chromophore induces remarkable beneficial effects on emission efficiency and photostability as compared to a tetramethylrhodamine analogue. We expect that the findings of the present study will be broadly applicable to the wide variety of fluorescent dyes, such as the dansyls, coumarins, and oxazines, as well as the numerous nonfluorescent classes of dyes that comprise the donor–acceptor family of chromogens. Work along these lines, in addition to extending the concepts that underscore the use of the 7-azanorbornyl prototype to higher order apex-substituted azabicycloalkane analogues, is currently in progress.

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**Supporting Information Available:** Preparation and characterization details for 221SR and TMSR, Tables of “Fluorescent lifetimes as a function of temperature” for 221SR and TMSR. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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